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Addressing Data Deficiencies in a Threatened Mobile Marine Predator

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ADDRESSING DATA DEFICIENCIES IN A THREATENED MOBILE MARINE PREDATOR

By

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ADDRESSING DATA DEFICIENCIES IN A THREATENED MOBILE MARINE PREDATOR

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DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship Declaration

I hereby declare that this dissertation incorporates material that is result of joint research, as follows: A.T. Fisk contributed to all chapters by providing the opportunities and facilities necessary to complete the research along with intellectual guidance. N.E. Hussey also contributed to all chapters by providing detailed comments and helpful direction. S.P. Wintner, G. Cliff and S. F.J. Dudley contributed to Chapter 2, 3, 4, and 6 by providing samples and helpful comments on the text. In Chapter 3, S.E. Campana contributed by providing data and comments on the manuscript. In Chapter 3, L.A. Kerr provided samples and comments on the manuscript. In Chapter 6, V. Lin, S. Tanaka, A. Velikanov, H.F. Mollet, and S.V. Fordham provided additional data and comments on the text. In all cases, the author, Heather M. Christiansen, performed the key ideas, data interpretation, and writing of all chapters.

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Dissertation Chapter	Publication title/full citation	Publication status*
Chapter 2	Christiansen HM, Hussey NE, Wintner SP, Cliff G, Dudley SFJ, Fisk AT (2014) Effect of sample preparation techniques for carbon and nitrogen stable isotope analysis of hydroxyapatite structures in the form of elasmobranch vertebral centra. Rapid Comm Mass Spec 28: 448-456	Published
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ABSTRACT

Knowledge of ecological and life-history traits over ontogeny for long-lived migratory marine species is vital to creating effective conservation and management strategies, however these details are often difficult to obtain. Incrementally growing biomineralized structures, such as elasmobranch vertebrae, are metabolically stable and provide the potential to investigate changes in spatial and trophic ecology over ontogeny. In the present dissertation, chemical tracer analysis ($\delta^{13}C$, $\delta^{15}N$, and $\Delta^{14}C$) and opportunistic sightings data are used to address data deficiencies for a threatened marine predator (white shark, *Carcharodon carcharias*).

Prior to the broad application of stable isotope analysis on elasmobranch vertebrae the methodological effect of demineralization on bulk $\delta^{13}C$ and $\delta^{15}N$ values was investigated. This study indicated that white and tiger (*Galeocerdo cuvier*) sharks do not require demineralization prior to analysis, allowing for fine-scale sampling over ontogeny. The age and growth parameters of white sharks off southern Africa were determined using bomb carbon and standard band counting method, revealing individuals are longer lived and slower growing than previously estimated for this region. Due to large variations in the ontogenetic profiles of stable isotopes among individual juvenile white sharks, it is recommended to integrate stable isotope data into a multidisciplinary framework to generate the most reliable data for resource managers. While the isotope values in the vertebrae of sub-adults revealed that individuals in this region have several strategies of habitat use and all individuals use habitat where no protection is currently in place (i.e., pelagic or north of KwaZulu-Natal along the east coast of Africa). Through a meta-analysis of opportunistic sightings and catch records of white sharks in the

northwest Pacific Ocean the temporal and spatial distribution patterns of individuals in this region were examined. These data included a large number of pregnant females and indicated the relative abundance of white sharks is relatively stable.

Through chemical tracer analysis and opportunistic sighting data this dissertation addressed knowledge gaps in the spatial ecology and life-history characteristics for a threatened species over ontogeny and advances our approaches to generate data to inform management on the ecological and life-history traits for difficult to study species.

DEDICATION

To Dan, Abigail, and Olivia,
Thank you for your constant love and support.

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CHAPTER 1: GENERAL INTRODUCTION

BACKGROUND AND RATIONAL

Nearly the entire ocean has been affected by human-mediated activities (Halpern et al. 2008) and there is mounting evidence that humans are a threat to the stability of species through over-fishing and habitat degradation (Hutchings 2000, Lotze et al. 2006, Polidoro et al. 2008, McClenachan et al. 2012, Polidoro et al. 2012). Sustainable management of fisheries is key to allowing for the continued exploitation of the world's oceans (Merino et al. 2012). While there have been success stories due to conservation (Butchart et al. 2006), the majority of species populations are continuing to worsen due to conservation efforts not offsetting threats such as habitat destruction, invasive species, and climate change (Hoffmann et al. 2010). Determining effective management of species is therefore a high priority to maintain the ocean's biodiversity (Merino et al. 2012).

The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species aims to provide rigorous evaluation of the status of plants and animals worldwide to guide conservation and management efforts. Currently, of the 41,517 described vertebrate species on the Red List, 7,781 are listed as threatened (including critically endangered, endangered, and vulnerable designations), with an additional 6,271 species listed as data deficient (IUCN, 2016). Conservation stakeholders require unambiguous data to assess a species risk level to formulate accurate species threat designations. The lack of comprehensive data for life-history characteristics and ecological traits of many species over ontogeny limits conservation efforts and effective management (Kinney and Simpfendorfer 2009, Knip et al. 2010, Simpfendorfer et al. 2011, Techara and Klein 2011). Understanding basic biology and ecology for all

populations and life stages of a species is therefore vital to developing effective conservation and management plans (Heppell et al. 2000, Mace et al. 2008, Simpfendorfer et al. 2011, Wallace et al. 2011). Thus, it has been recognized that research for data deficient species should be a high priority to determine true threat status (Butchart and Bird 2010).

MIGRATORY MARINE ANIMALS

Conservation of migratory marine predators is critical as they fill an important role within ocean ecosystems through influence on prey species and maintaining ecosystem stability (Scheffer et al. 2005, Block et al. 2011). Determining traits such as habitat use, movement, and migration patterns is key to developing effective management plans (Ramos and González-Solís 2012). Conservation of highly migratory marine species that cross international boundaries is complicated requiring international cooperation to manage the species (Shillinger et al. 2008, Speed et al. 2010, Block et al. 2011). Additionally, for species that are globally distributed regional populations may face different threats and pressures or have variation in life-history and ecological traits requiring region specific management (Bowen et al. 2005, Wallace et al. 2010).

Many elasmobranch species (sharks and rays) are migratory and have a wide range of life-history and ecological traits (Compagno 2001, Camhi 2008). Of the 1,041 chondrichthyan species assessed by Dulvy et al. (2014), it was found that 181 (17.4%) are classified as threatened, with an additional 487 (46.8%) classified as data deficient. Many shark species are at risk due to threats from bycatch (Molina and Cooke 2012) and their low recovery potential (Smith et al. 1998, Ward-Paige et al. 2012) and large population declines have been observed (Stevens et al. 2000, Dulvy et al. 2008, Worm et

al. 2013). In a review of conservation priorities, Simpfendorfer et al. (2011) identified 26 areas of research needs for elasmobranchs including details on age and growth parameters, life-history, spatial and trophic ecology, and ecosystem role.

The removal of top predators from marine ecosystems has been documented to have cascading effects (Camhi 2008, Baum and Worm 2009) emphasizing the need for effective management strategies to be implemented for large species such as sharks. Reef sharks are typically considered apex predators in coral reef systems; however, when incorporating stomach content data and stable isotope analysis a recent study showed reef sharks function similar to large predator fishes (Frisch et al. 2016). Alternatively, the complex interactions and trophic position of large sharks off southern Africa have been underestimated by assuming functional equivalency with large predatory fish (Hussey et al. 2015). The potential negative effect of management decisions based on incomplete data was highlighted in a recent study by Grubbs et al. (2016), whereby a classic example of a trophic cascade described by Myers et al. (2007) was shown to be unsubstantiated after examination of additional data. Management decisions based on the Myers et al. (2007) study increased fishing pressure on the slow-growing cownose ray (*Rhinoptera bonasus*) potentially having negative consequences on the population's stability. These studies stress the need for more complete data on life-history and ecological trends to inform management before broadly assuming the trophic role of large predators in their surrounding ecosystem.

DISSERTATION OBJECTIVE

The objective of this dissertation is to address stage specific data deficiencies in the knowledge of life-history characteristics, spatial, and foraging ecology for a

threatened marine species. This dissertation seeks to advance our approaches in providing data used to inform and create effective management plans across a range of threatened species. Using white sharks, *Carcharodon carcharias*, as a model species this dissertation uses a combination of chemical tracers and meta-analysis to provide details on life-history and ecological traits within and among individuals.

CHARACTERIZING INDIVIDUAL AND POPULATION LEVEL TRAITS

Traditional methods to investigate life-history characteristics and ecological traits in migratory marine animals such as tag and release using standard, acoustic, and satellite tags (Luschi et al. 1998, Smith et al. 2003, Speed et al. 2010, Block et al. 2011), photographic-identification (Smith et al. 1999, Arzoumanian et al. 2005, Couturier et al. 2011, Towner et al. 2013), and opportunistic sampling (Mignucci-Giannoni et al.1999, Wintner and Cliff 1999, Dudley and Simpfendorfer 2006, Cliff and Dudley 2011) provide valuable data at the individual level. However, the data collected by these methods are often limited in time frame by size of the organism or require re-sightings or recaptures of individuals to obtain the data. Due to changes in ecological strategies undertaken by individuals over ontogeny, there is also the need to examine each life stage for an individual.

The use of chemical tracers in ecological studies has increased substantially over the last 20 years (Inger and Bearhop 2008, Martínez del Rio et al. 2009). Chemical tracers provide several advantages over traditional tagging methods to study ecological traits, as they are ubiquitous throughout the environment allowing all individuals in a population to be tagged without prior capture. Additionally, there is no alteration of behavior due to capture. Perhaps the most advantageous trait to chemical tracer analysis

is the prospect of retrospective analysis that can provide data over an individual's life; if sufficient sample sizes are available generalizations at the population level, can be made to assist conservation managers in making informed decisions.

Chemical tracers such as the stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) can for example, be used as natural tags to provide insight on the spatial use and foraging ecology of a species (Peterson and Fry 1987, Hobson 1999). Relative trophic position in a food web can be inferred based on the predictable enrichment between predator and prey and the $\delta^{15}N$ value of an organism (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987, Post 2002, Hussey et al. 2014). The enrichment of ^{13}C is typically more conservative between trophic levels and provides a measure of the primary productivity at the base of the food chain where the organism feeds and is consequently used as an indication of foraging location and habitat usage (DeNiro and Epstein 1978, Peterson and Fry 1987).

Bomb radiocarbon (^{14}C) dating has been used to validate ageing protocols in marine structures throughout the world's oceans (Kalish 1993, Campana 1999, Goldman et al. 2012). As a result of atmospheric testing of thermonuclear devices in the 1950s and 1960s the amount of radiocarbon in the world's oceans rapidly increased (Druffel and Linick 1978, Broecker and Peng 1982). The uptake of radiocarbon in marine carbonates was nearly synchronous and created a dated reference for year of formation in accretionary structures. Therefore, for species whose ageing protocol has not been validated the $\Delta^{14}C$ values of an individual can be compared to a reference chronology to confirm the year of formation for each sampling location.

Investigating changes in the chemical composition of biomineralized structures

(e.g. fish otoliths, corals, mollusc shells) over ontogeny has provided details on age, habitat use, migration, and diet (Stecher et al. 1996, Sinclair et al. 1998, Campana 1999, Elsdon et al. 2008). Elasmobranch vertebrae are biomineralized structures formed by accretionary growth (Weiner and Dove, 2003), and both the organic (Campana et al. 2002) and inorganic (Tillett et al. 2011, Smith et al. 2013) portions of elasmobranch vertebrae have been determined to be metabolically stable. Many elasmobranch species are long-lived with stage specific ecological traits and life-history characteristics; therefore using elasmobranch vertebrae allows for retrospective analysis to be conducted providing data for each life stage of an individual, while the number of vertebrae in archived and museum collections provide samples to examine population level data.

Vertebral samples for Chapters 2-5 were obtained from the KwaZulu-Natal Sharks Board (KZNSB), KwaZulu-Natal, South Africa. The KZNSB maintains a series of beach protection nets and drum lines along the eastern coastline of South Africa (Cliff and Dudley 2011). Specimens found deceased, but in good condition are taken to the laboratory for biological sampling. These dissections have resulted in a long lasting archive of biological samples and data for shark species off South Africa's eastern coast (Cliff and Dudley 1992, Dudley and Simpfendorfer 2006).

MODEL SPECIES

The white shark (*Carcharodon carcharias*) is a cosmopolitan species throughout the world's temperate and tropical oceans (Compagno 2001). Based on preliminary research white sharks were protected in South Africa in 1991 (Compagno 1991). Since this time white sharks have been listed as vulnerable on the IUCN Red List, on Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and

Flora (CITES) and on both Appendices I and II of the Convention on Migratory Species (CMS). Protection to white sharks has also been extended in Australia, Canada (Atlantic Ocean), Croatia, European Union, Maldives, Malta, Mexico, Namibia, New Zealand, South Africa, and in all United States waters (except in the Western Pacific) (Camhi et al. 2009, Kyne et al. 2012).

There are several white shark aggregation sites worldwide and knowledge of spatial ecology and life-history characteristics vary by region (Bruce 2008, Domeier 2012, Dudley 2012). Movement strategies and residency patterns in white sharks can vary based on region, size, and sex (Ferreira and Ferreira 1996, Bonfil et al. 2005, Bruce and Bradford 2012, Duffy et al. 2012, Jorgensen et al. 2012). White sharks function as top predators throughout their range with an observed diet shift with increasing body size to incorporate marine mammals (Tricas and McCosker 1984, Hussey et al. 2012). Genetic evidence has shown that white shark regional populations vary genetically (Pardini et al. 2001, Jorgensen et al. 2010, Tanaka et al. 2011, Gubili et al. 2012, O'Leary et al. 2015). Due to the differences in genetic, ecological, and life-history traits observed among populations region specific information is required to aid in conservation and management efforts. I focus on the population of white sharks in the southwest Indian Ocean to examine the potential of using chemical tracers as a tool to investigate spatial and trophic ecology over the lifetime of an individual to inform management at the population level. To examine how knowledge can be advanced on key ecological traits of these threatened species without physical samples and chemical analyses, I also conduct a meta-analysis on the white shark population in the northwest Pacific Ocean.

OVERVIEW OF CHAPTERS

This dissertation aims to examine life-history characteristics and habitat use of a threatened species both at the individual and population level following key research areas identified by Simpfendorfer et al. (2011) (Figure 1.1). Addressing several assumptions made for stable isotope analysis of elasmobranch vertebrae (Chapter 2) lays the foundation for ontogenetic stable isotope analysis (Chapter 4 and 5). While life-history characteristics are investigated in Chapters 3 and 6. Additionally, Chapter 6 provides key data on the spatial and temporal distribution of a lesser-studied population. Data generated by this dissertation will aid in refining current management of a threatened species and provide support for further international cooperation in the management of this species.

I examine the processes and mechanisms regulating bulk stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of elasmobranch vertebrae in Chapter 2 (Effect of sample preparation techniques for carbon and nitrogen stable isotope analysis of hydroxyapatite structures in the form of elasmobranch vertebral centra). Carbohydrates, lipids, and protein supply carbon in the inorganic portion of the vertebrae whereas; the carbon in the organic portion (collagen) is sourced from protein only (Newsome et al. 2010, Kim and Koch 2012). As the proportion of inorganic material varies throughout the vertebrae it is possible that the bulk $\delta^{13}C$ value may be biased by the inorganic contribution. It has been suggested that vertebral samples should be treated with ethylenediaminetetraacetic acid (EDTA) prior to stable isotope analysis to obtain an unbiased measure of $\delta^{13}C$ (Kim and Koch 2012). In this chapter, I examine the effect of EDTA treatment on vertebral stable isotope values from three species of sharks (sand tiger, *Carcharias taurus*; tiger,

Galeocerdo cuvier; and white sharks). The minimum starting sample weight required to obtain reliable and consistent stable isotope values will also be determined, as the temporal range of a sample (i.e., time period the sample is representative of) is limited by how much sample material is required. This chapter standardizes the sample preparation method of isotope analysis in elasmobranch vertebrae, which will be used in subsequent chapters.

Bomb radiocarbon analysis ($\Delta^{14}C$) is used in chapter three (Using bomb radiocarbon to validate age and estimate growth of the white shark, *Carcharodon carcharias*, from the southwestern Indian Ocean), to investigate the age and growth of white sharks from the southwestern Indian Ocean. Elasmobranch vertebrae grow incrementally and annual growth rings (consisting of one opaque and translucent band) can be used to estimate age of elasmobranch species (Cailliet and Goldman, 2004). However, growth bands are not annual for all species (Natanson and Cailliet 1990, Natanson et al. 2008, Huveneers et al. 2013) or populations of a species (Campana et al. 2002, Francis et al. 2007) and therefore must be validated for each species, population and size range (Beamish and McFarlane 1983, Cailliet et al. 2006). I predict that the sudden increase of radiocarbon in the world's oceans will be reflected in the $\Delta^{14}C$ values of white shark vertebrae from southern Africa and can be used to validate the band counting technique for this species. Furthermore, I predict that the growth rates for white sharks will be similar to those determined for white sharks in the northwest Atlantic Ocean. This chapter provides data on age and growth rates for a threatened species; which are important for helping define management strategies and set sustainable fishing limits.

The current paradigm for young-of-the-year and juvenile habitat use is that there is a single nursery located in the Eastern Cape of South Africa. This has been perpetuated due to the observations of small individuals (~150 cm) in this region in this region (Cliff et al. 1996, Dicken 2008). However, juvenile white sharks are documented to frequently occur in KwaZulu-Natal (Cliff et al. 1989, Dudley 2012) and are thought to be generalist predators based on stomach content analysis (Hussey et al. 2012). Therefore, I predict that vertebral stable isotope profiles for juvenile white sharks will be similar and have little variation among individuals. In chapter 4 (Incorporating stable isotopes into a multidisciplinary framework to improve data inference and their conservation and management application), I explore the causes of stable isotope variation ($\delta^{13}C$ and $\delta^{15}N$) within and among juvenile white sharks and the implications each possible explanation has for management. I suggest that stable isotope data be incorporated with multidisciplinary methods (e.g. genetics, telemetry, etc.) to generate the most reliable data for conservationists and resource managers. If this is not possible, I suggest that multiple plausible explanations for variation in data be provided rather than focusing on a single explanation that could potentially misguide management.

I investigate the habitat use of sub-adult white sharks and how it varies both within and among individuals in Chapter 5 (Examining habitat use and movements within and among sub-adult white sharks (*Carcharodon carcharias*) off southern Africa). Gradients in stable isotope values along the coast of South Africa and extending offshore (Hill et al. 2006, Hill and McQuaid 2008) allow for the characterization of distinct regional prey isotope values (i.e., temperate, tropical, and pelagic). Using a Bayesian mixing model, MixSIAR (Stock and Semmens 2015), the contribution of regional prey

groups to an individual shark's diet is estimated and then used as a proxy for habitat use. Vertebrae were serially sampled to provide details on how an individual's diet and habitat change over time. Current research suggests that there are aggregations of sub-adult white sharks at seal colonies in the Western Cape and along the KwaZulu-Natal coast (Dudley 2012). Bonfil et al. (2005) reported white sharks that undertook oceanic migrations, however the frequency or extent of these migrations is currently unknown. Based on electronic tagging studies (Bonfil et al. 2005), I hypothesize individual sharks will obtain the majority of their prey resources from the Eastern and Western Cape as sub-adults. While white sharks are protected in South Africa, there is no protection along the east coast of Africa (from Mozambique northwards), therefore it is important to examine if white sharks are regularly using this habitat. This chapter will provide details on regional habitat use within the white shark population off southern Africa. Further, it will provide details on how habitat use varies within and among individuals. These details will help inform management on whether current strategies protecting white sharks in the southwest Indian Ocean are sufficient or if more protection is required in the surrounding seas and or along the African coastline.

I investigate spatial ecology and life-history characteristics for a data deficient sub-population in Chapter 6 (The last frontier: catch records of white sharks (*Carcharodon carcharias*) in the northwest Pacific Ocean). Globally distributed species may require region-specific management protocols due to differing threats and pressures by region (Halpern et al. 2007, Wallace et al. 2010). I will conduct a meta-analysis on catch records and observations obtained from several sources (i.e., scientific literature, fisheries bycatch, media accounts, personal observations, etc.). This chapter will provide

details on the spatial and temporal distribution of a threatened species in an under-studied region, which is required to guide future research and monitoring, and will assist regional conservation management.

Finally, in chapter 7, I will summarize the chapters presented here and highlight the data deficiencies that have been addressed within this dissertation. I will discuss using chemical tracer techniques, such as carbon and nitrogen stable isotopes and bomb radiocarbon, to generate data to serve as a guide on how to process and analyze data in other species that have biomineralized structures and how to gather data in data poor regions. The overall contributions this dissertation has made to advance our knowledge of life-history characteristics and spatial ecology for a threatened marine predator and directions for future research will be discussed.

REFERENCES

Arzoumanian Z, Holmberg J, Norman B (2005) An astronomical pattern-matching algorithm for computer-aided identification of whale sharks *Rhincodon typus*. J Appl Ecol 42: 99-1011

Baum JK, Worm B (2009) Cascading top-down effects of changing oceanic predator abundances. J Anim Ecol 78: 699–714

Beamish RJ, McFarlane GA (1983) The forgotten requirement for age validation in fisheries biology. Trans Am Fish Soc 112: 735–743

Block BA, Jonsen ID, Jorgensen SJ, Winship AJ, Shaffer SA et al. (2011) Tracking apex marine predator movements in a dynamic ocean. Nature 475: 86-90

Bonfil R, Meyer M, Scholl MC, Johnson R, O'Brien S et al. (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310: 100-103

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Mol Ecol 14: 2389–2402

Broecker WS, Peng TH (1982) Tracers in the Sea. Lamont-Doherty Geological Observatory: Palisades, New York. 690 p.

Bruce BD (2008) The biology and ecology of the white shark *Carcharodon carcharias* In: Camhi MD, Pikitch EK, Babcock EA (eds) Sharks of the Open Ocean: Biology, Fisheries and Conservation. Blackwell Publishing, Oxford, U.K. pp 69-81

Bruce BD, Bradford RW (2012) Habitat use and spatial dynamics of juvenile white sharks, *Carcharodon carcharias*, in Eastern Australia. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 225-253

Butchart SHM, Bird JP (2010) Data deficient birds on the IUCN Red List: What don't we know and why does it matter? Biol Cons 143: 239-247

Butchart SHM, Stattersfield AJ, Collar NJ (2006) How many bird extinctions have we prevented? Oryx 40: 266-278

Cailliet GM, Goldman KJ (2004) Age determination and validation in chondrichthyan fishes. In: Carrier JC, Musick JA, Heithaus MR (eds) Biology of Sharks and their Relatives. CRC Press, New York, pp 399-447

Cailliet GM, Smith WD, Mollet HF, Goldman KJ (2006) Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. Environ Biol Fish 77: 211-228

Camhi MD (2008) Conservation status of pelagic elasmobranchs. In: Camhi MD, Pikitch EK, Babcock EA (eds) Sharks of the Open Ocean: Biology, Fisheries and Conservation. Blackwell Publishing, Oxford, U.K. pp 397-417

Camhi MD, Valenti SV, Fordham SV, Fowler SL, Gibson C (2009) The conservation status of pelagic sharks and rays: report of the IUCN Shark Specialist Group Pelagic Shark Red List Workshop. IUCN Species Survival Commission Shark Specialist Group. Newbury, UK 78 p.

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188: 263-297

Campana SE, Natanson LJ, Myklevoll S (2002) Bomb dating and age determination of large pelagic sharks. Can J Fish Aquat Sci 59: 450-455
Cliff G, Dudley SFJ (1992) Protection against shark attack in South Africa, 1952 to 1990. In: Pepperell JG (ed) Sharks: Biology and Fisheries. Aus J Mar Freshw Res 43: 263-272
Cliff G, Dudley SFJ (2011) Reducing the environmental impact of shark control programs: a case study from KwaZulu-Natal, South Africa. Mar Freshw Res 62: 700-709
Cliff G, Dudley SFJ, Jury MR (1996) Catches of white sharks in KwaZulu-Natal, South Africa and environmental influences. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 351-362
Compagno LJV (1991) Government protection for the great white shark (*Carcharodon carcharias*) in South Africa. S Afr J Sci 87: 284-285
Compagno LJV (2001) Sharks of the world: An annotated and illustrated catalogue of shark species known to date. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Cat Fish Purposes No. 1 2: 1-269
Couturier LIE, Jaine FRA, Townsend KA, Weekes SJ, Richardson AJ et al. (2011) Distribution, site affinity and regional movements of the manta ray, *Manta alfredi* (Krefft 1868) along the east coast of Australia. Mar Freshw Res 62: 628–637
DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495-506
DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341-351
Dicken ML (2008) First observations of young of the year and juvenile great white sharks (*Carcharodon carcharias*) scavenging from a whale carcass. Mar Freshw Res 59: 596-602
Domeier ML (2012) A new life-history hypothesis for White Sharks, *Carcharodon carcharias*, in the Northeastern Pacific. In: Domeier ML(ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 199-223
Druffel EM, Linick TW (1978) Radiocarbon in annual coral rings of Florida. Geophys Res Lett 5: 913–916
Dudley SFJ (2012) A review of research on the white shark, *Carcharodon carcharias*, in southern Africa. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 511-533
Dudley SFJ, Simpfendorfer CA (2006) Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. Mar Freshw Res 57: 225-240
Duffy CAJ, Francis MP, Manning MJ, Bonfil R (2012) Regional population connectivity, oceanic habitat, and return migration revealed by satellite tagging of white sharks, *Carcharodon carcharias*, at New Zealand aggregation sites. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 301-318

Dulvy NK, Baum JK, Clarke S, Compagno LJV, Cortés E et al. (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquat Conserv 18: 459-482
Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM et al. (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3: e00590
Elsdon TS, Wells BK, Campana SE, Gillanders BM, Jones CM et al. (2008) Otolith chemistry to describe movements and life history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanogr Mar Biol 46: 297-330
Ferreira CA, Ferreira TP (1996) Population dynamics of white sharks in South Africa. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias.* Academic Press, San Diego, pp 381-391
Francis MP, Campana SE, Jones CM (2007) Age under-estimation in New Zealand porbeagle sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? Mar Freshw Res 58: 10-23
Frisch AJ, Ireland M, Rizzari JR, Lönnstedt OM, Magnenat KA et al. (2016) Reassessing the trophic role of reef sharks as apex predators on coral reefs. Coral Reefs 1-14 doi:10.1007/s00338-016-1415-2
Goldman KJ, Cailliet GM, Andrews AH, Natanson LJ (2012) Assessing the age and growth of chondrichthyan fishes. In: Carrier J, Musick JA, Heithaus MR (eds) Biology of Sharks and their Relatives. CRC Press, Boca Raton, pp 423- 451
Grubbs RD, Carlson JK, Romine JG, Curtis TH, McElroy WD et al. (2016) Critical assessment and ramifications of a purported marine trophic cascade. Sci Rep 6:20970
Gubili C, Duffy CAJ, Cliff G, Wintner SP, Shivji M et al. (2012) Application of molecular genetics for conservation of the white shark, *Carcharodon carcharias,* L. 1758. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 357-380
Halpern BS, Selkoe KA, Micheli F, Kappel CV (2007) Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Conserv Biol 21: 1301-1315
Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F et al. (2008) A global map of human impact on marine ecosystems. Science 319: 948-952
Heppell SS, Caswell H, Crowder LB (2000) Life histories and elasticity patterns: Perturbation analysis for species with minimal demographic data. Ecology 81: 654-665
Hill JM, McQuaid CD (2008) $\delta^{13}C$ and $\delta^{15}N$ biogeographic trends in rocky intertidal communities along the coast of South Africa: Evidence of strong environmental signatures. Estuar Coast Shelf S 80: 261–268
Hill JM, McQuaid CD, Kaehler S (2006) Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of Southern Africa. Mar Ecol Prog Ser 318: 63–73
Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314-326
Hoffman M, Hilton-Talor C, Angulo A, Böhm M, Brooks TM et al. (2010) The impact of conservation on the status of the world's vertebrates. Science 330: 1503-1509
Hussey NE, McCann HM, Cliff G, Dudley SFJ, Wintner SP et al. (2012) Size-based

analysis of diet and trophic position of white shark (*Carcharodon carcharias*) in South African water. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 27-49
Hussey NE, MacNeil MA, McMeans BC, Olin JA, Dudley SFJ et al. (2014) Rescaling the trophic structure of marine food webs. Ecol Lett 17: 239-250
Hussey NE, MacNeil MA, Siple MC, Popp BN, Dudley SFJ et al. (2015) Expanded trophic complexity among large sharks. Food Webs 4: 1-7
Hutchings JA (2000) Collapse and recovery of marine fishes. Nature 406: 882–885
Huveneers C, Stead J, Bennett MB, Lee KA, Harcourt RG (2013) Age and growth determination of three sympatric wobbegong sharks: How reliable is growth band periodicity in Orectolobidae? Fish Res 147: 413-425
Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. Ibis 150: 447-461
IUCN Red List of Threatened Species. Version 2015-4. <www.iucnredlist.org>. Downloaded on 01 February 2016
Jorgensen SJ, Reeb CA, Chapple TA, Anderson S, Perle C et al (2010) Philopatry and migration of Pacific white sharks. Proc R Soc B 277: 679-688
Jorgensen SJ, Chapple TK, Anderson S, Hoyos M, Reeb C et al. (2012) Connectivity among white shark coastal aggregation areas in the northeastern Pacific. In: Domeier M (ed) Global Perspectives on the Biology and Life History of the Great White Shark (*Carcharodon carcharias*). CRC Press, Boca Raton, pp 159-167
Kalish JM (1993) Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet Sci Lett 114: 549-554
Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fish 95: 53-63
Kinney MJ, Simpfendorfer CA (2009) Reassessing the value of nursery areas to shark conservation and management. Conserv Lett 2: 53-60
Knip DM, Heupel MR, Simpfendorfer CA (2010) Sharks in nearshore environments: models, importance, and consequences. Mar Ecol Prog Ser 402: 1-11
Kyne PM, Carlson JK, Ebert DA, Fordham SV, Bizzarro JJ et al. (eds), (2012) The Conservation Status of North American, Central American, and Caribbean Chondrichthyans. IUCN Species Survival Commission Shark Specialist Group, Vancouver, Canada.
Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG et al. (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312: 1806-1809
Luschi P, Hays GC, del Seppia C, Marsh R, Papi F (1998) The navigational feats of green sea turtles migrating from ascension island investigated by satellite telemetry. Proc R Soc Lond B 265: 2279-2284
Mace GM, Collar NJ, Gaston KJ, Hilton-Taylor C, Akçakaya HR et al. (2008) Quantification of extinction risk: IUCN's system for classifying threatened species. Conserv Biol 22: 1424-1442
Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84: 91-111
McClenachan L, Cooper AB, Carpenter KE, Dulvy NK (2012) Extinction risk and

bottlenecks in the conservation of charismatic marine species. Cons Lett 5: 73–80
Merino G, Barange M, Blanchard JL, Harle J, Holmes R et al. (2012) Can marine fisheries and aquaculture meet fish demand from a growing human population in a changing climate? Global Environ Change 22: 795-806
Mignucci-Giannoni AA, Toyos-González GM, Pérez-Padilla J, Rodríguez-López MA, Overing J (1999) Mass stranding of pygmy killer whales (*Feresa attenuata*) in the British Virgin Islands. J Mar Biol Ass UK 79: 383-384
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48: 1135-1140
Molina JM, Cooke SJ (2012) Trends in shark bycatch research: current status and research needs. Rev Fish Biol Fisheries 22: 719-737
Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315: 1846–1850
Natanson LJ, Cailliet GM (1990) Vertebral growth zone deposition in Pacific angel sharks. Copeia 4: 1133-1145
Natanson LJ, Wintner SP, Johansson F, Piercy A, Campbell P et al. (2008) Ontogenetic vertebral growth patterns in the basking shark *Cetorhinus maximus*. Mar Ecol Prog Ser 361: 267-278
Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci 26: 509-572
O'Leary SJ, Feldheim KA, Fields AT, Natanson LJ, Wintner SP et al (2015) Genetic diversity of white sharks, *Carcharodon carcharias*, in the northwest Atlantic and southern Africa. J Hered 106: 258-265
Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H et al (2001) Sex-biased dispersal of great white sharks. Nature 412: 139-140
Peterson B, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320
Polidoro BA, Livingstone SR, Carpenter KE, Hutchinson B, Mast RB et al. (2008) Status of the world's marine species. In: Vié JC, Hilton-Taylor C, Stuart SN (eds) Wildlife in a changing world: an analysis of the 2008 IUCN Red List of threatened species. Gland, Switzerland: International Union for Conservation of Nature, pp 55-65
Polidoro BA, Brooks T, Carpenter KE, Edgar GJ, Henderson S et al. (2012) Patterns of extinction risk and threat for marine vertebrates and habitat-forming species in the Tropical Eastern Pacific. Mar Ecol Prog Ser 448: 93-104
Post DL (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718
Ramos R, González-Solís (2012) Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. Front Ecol Environ 10: 258-266
Scheffer M, Carpenter S, de Young B (2005) Cascading effects of overfishing marine systems. Trends Ecol Evol 20: 579–581
Shillinger GL, Palacios DM, Bailey H, Bograd SJ, Swithenbank AM et al. (2008) Persistent leatherback turtle migrations present opportunities for conservation. PLoS Biology 6: 1408-1416

Simpfendorfer CA, Heupel MR, White WT, Dulvy NK (2011) The importance of research and public opinion to conservation management of sharks and rays: a synthesis. Mar Freshw Res 62: 518-527
Sinclair DJ, Kinsley LPJ, McCulloch MT (1998) High resolution analysis of trace elements in corals by laser ablation ICP-MS. Geochim Cosmochim Acta 62: 1889-1901
Smith SE, Au DW, Show C (1998) Intrinsic rebound potentials of 26 species of Pacific sharks. Mar Freshw Res 49: 663-678
Smith SE, Mitchell RA, Fuller D (2003) Age-validation of a leopard shark (*Triakis semifasciata*) recaptured after 20 years. Fish Bull 101: 194-198
Smith TD, Allen J, Clapham PJ, Hammond PS, Katona S et al (1999) An ocean-basin-wide mark-recapture study of the North Atlantic humpback whale (*Megaptera novaeangliae*). Mar Mamm Sci 15: 1-32
Smith WD, Miller JA, Heppell SS (2013) Elemental markers in elasmobranchs: effects of environmental history and growth on vertebral chemistry. PLoS ONE 8: e62423
Speed CW, Field IC, Meekan MG, Bradshaw CJA (2010) Complexities of coastal shark movements and their implications for management. Mar Ecol Prog Ser 208: 275-305
Stecher III HA, Krantz DE, Lord III CJ, Luther III GW, Bock KW (1996) Profiles of strontium and barium in *Mercenaria mercenaria* and *Spisula solidissima* shells. Geochim Cosmochim Acta 60: 3445-3456
Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES J Mar Sci 57: 476-494
Stock BC, Semmens BX (2015) MixSIAR User Manual, version 3.0. https://github.com/brianstock/MixSIAR/
Tanaka S, Kitamura T, Mochizuki T, Kofuji K (2011) Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kashima-nada, Japan. Mar Freshw Res 62: 548-556
Techera EJ, Klein N (2011) Fragmented governance: reconciling legal strategies for shark conservation and management. Mar Policy 35: 73–78
Tillett BJ, Meekan MG, Parry D, Munksgaard N, Field IC et al. (2011) Decoding fingerprints: elemental composition of vertebrae correlates to age-related habitat use in two morphologically similar sharks. Mar Ecol Prog Ser 434: 133-142
Towner AV, Wcisel MA, Reisinger RR, Edwards D, Jewell OJD (2013) Gauging the threat: The first population estimate for white sharks in South Africa using photo identification and automated software. PLoS ONE 8(6): e66035
Tricas TC, McCosker JE (1984) Predatory behavior of the white shark, *Carcharodon carcharias*, with notes on its biology. Proc Cal Acad Sci 14: 221-238
Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB et al. (2010) Regional management units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. PLoS ONE 5(12): e15465
Wallace BP, DiMatteo AD, Bolten AB, Chaloupka MY, Hutchinson BJ et al. (2011) Global conservation priorities for marine turtles. PLoS ONE 6(9): e24510
Ward-Paige CA, Keith DM, Worm B, Lotze HK (2012) Recovery potential and conservation options for elasmobranch. J Fish Biol 80: 1844-1869

Weiner S, Dove PM (2003) An overview of biomineralization processes and the problem of the vital effect. In: Dove PM, DeYoreo JJ, Weiner S, (eds) Biomineralization. pp 1-29

Wintner SP, Cliff G (1999) Age and growth determination of the white shark, *Carcharodon carcharias*, from the east coast of South Africa. Fish Bull 97: 153-169

Worm B, Davis B, Kettemer L, Ward-Page CA, Chapman D et al. (2013) Global catches, exploitation rates, and rebuilding options for sharks. Mar Policy 40: 194-204

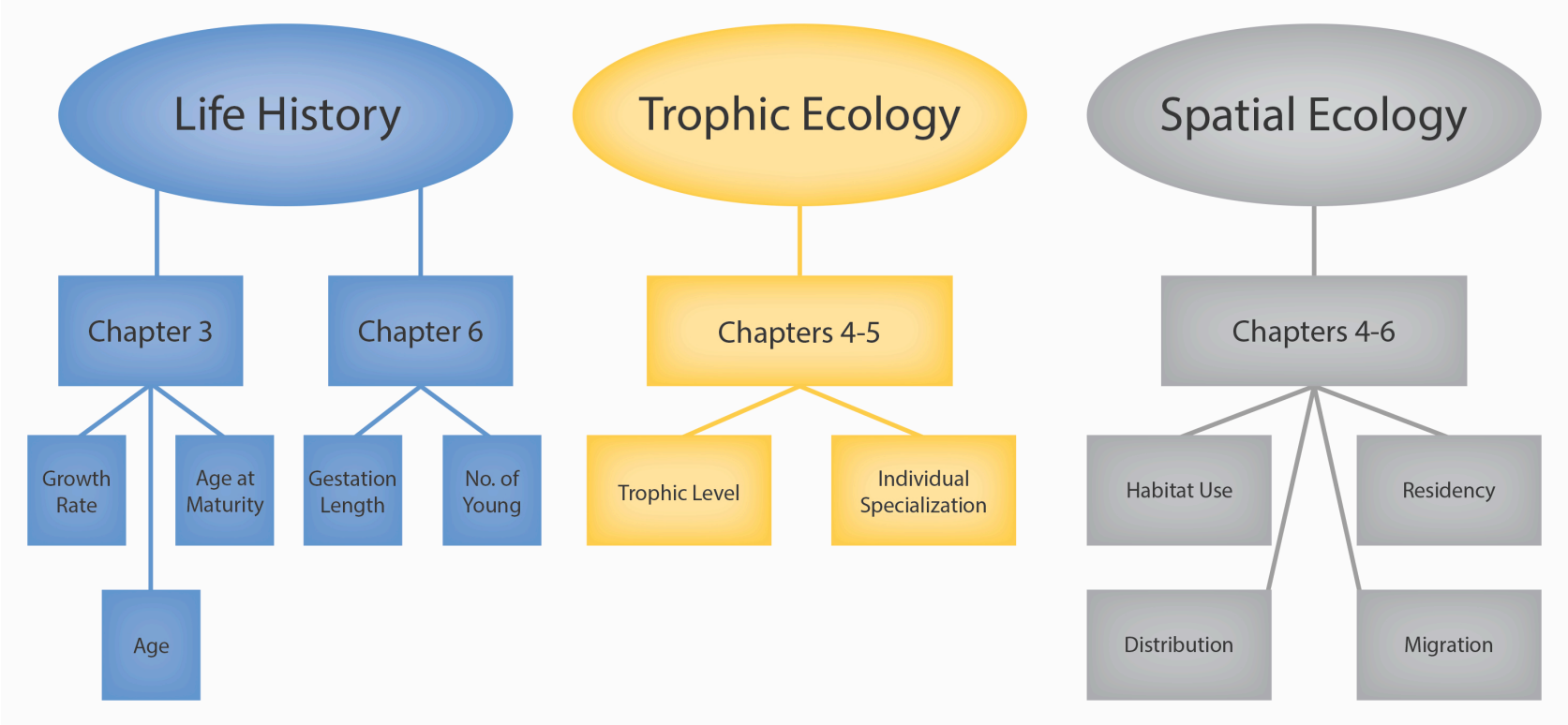

Figure 1.1 Chapter outlines of specific data deficiencies addressed for the white shark (*Carcharodon carcharias*) within this dissertation.

CHAPTER 2: EFFECT OF SAMPLE PREPARATION TECHNIQUES FOR CARBON AND NITROGEN STABLE ISOTOPE ANALYSIS OF HYDROXYAPATITE STRUCTURES IN THE FORM OF ELASMOBRANCH VERTEBRAL CENTRA[1]

INTRODUCTION

Bulk stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in animal tissues is a common tool used to study the movement, foraging, and trophic ecology of species, including elasmobranchs (sharks, skates, and rays) (Hussey et al. 2012). For most organisms, the $\delta^{13}C$ value of a tissue has been shown to reflect diet and primary productivity (i.e. indicative of foraging base) (DeNiro and Epstein 1978, Peterson and Fry 1987) and is often used as a chemical tracer of species movement (Best and Schell 1996, Hobson 1999). The $\delta^{15}N$ values exhibit a more marked increase between predator and prey, such that relative trophic position and feeding behavior of the individual can be inferred (DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987). Combined quantitative analysis of $\delta^{13}C$ and $\delta^{15}N$ data for single or multiple species has led to novel insights into seasonal migrations (Best and Schell 1996), dietary specialization within a population (Newsome et al. 2009, Vander Zanden et al. 2010), and community-wide trophic structure (Layman et al. 2007).

Standardized sample storage and preparation methods prior to stable isotope analysis are an important consideration to ensure consistent results among studies and populations. For elasmobranch muscle tissue the effects of ethanol preservation and lipid extraction have been found to alter $\delta^{13}C$ and $\delta^{15}N$ values, respectively (Hussey et al. 2012, Kim and Koch 2012). Inconsistent effects on stable isotope values following storage and treatment methods indicates these factors need to be addressed on an

[1] Christiansen HM, Hussey NE, Wintner SP, Cliff G, Dudley SFJ, Fisk AT (2014) Effect of sample preparation techniques for carbon and nitrogen stable isotope analysis of hydroxyapatite structures in the form of elasmobranch vertebral centra. Rapid Comm Mass Spec 28: 448-456

individual tissue and species basis. Tissues such as blood and muscle can be obtained non-lethally, providing valuable data for an individual, but typically represent a single snap shot value and, because of relatively fast tissue turnover rates, indicate only recent feeding behavior (MacNeil et al. 2005, Matich et al. 2011). Biomineralized structures in vertebrate species (e.g., whale baleen, turtle scutes, and elasmobranch vertebrae) are metabolically inert (Best and Schell 1996, Campana et al. 2002, Vander Zanden et al. 2010) and have the potential to provide important and novel ecological information, but have received less attention (Best and Schell 1996, Newsome et al. 2009, Vander Zanden et al. 2010). Elasmobranch vertebrae are biomineralized structures, which form annual growth rings in many species (Cailliet and Goldman 2004), that can be individually sampled to provide an isotopic history throughout ontogeny (including pre-natal information formed during development).

Elasmobranch vertebral centra consist of an inorganic (mineralized) portion in the form of areolar calcification (Dean and Summers 2006), and an organic portion (collagen), but also include proteoglycans and water (Porter et al. 2006). The inorganic portion may contain a different $\delta^{13}C$ value from the organic portion of interest, which can result in a mixed measured $\delta^{13}C$ value (Larson et al. 2008, Kim and Koch 2012), an effect previously reported in terrestrial and marine mammal bones (Newsome et al. 2010). A common technique to address this issue is to decalcify the structure using hydrochloric acid (HCl), thus removing the inorganic portion, prior to bulk stable isotope analysis (Jacob et al. 2005, Carabel et al. 2006, Brodie et al. 2011a). In the first isotopic studies on elasmobranch vertebrae multiple preparation methods were used prior to analysis including drying samples (Estrada et al. 2006) and decalcifying by HCl with lipid

extraction (Kerr et al. 2006). In an attempt to standardize these pre-treatment methods, Kim and Koch (2012) suggested isolating the collagen in elasmobranch vertebrae through treatment with ethylenediaminetetraacetic acid (EDTA), a method that has successfully been used to isolate collagen in human bone (Tuross et al. 1988) and ostrich eggshells (Johnson et al. 1998). Kim and Koch (2012) suggested that EDTA was a more suitable method for removing the inorganic component of elasmobranch vertebrae rather than HCl due to rapid decalcification when using the latter. Consequently, a higher percentage yield of collagen is retained in samples treated with EDTA versus those treated with HCl, which is beneficial when only small amounts of material are available for analysis (Tuross et al. 1988, Kim and Koch 2012).

A key advantage of using elasmobranch vertebrae for stable isotope analysis is the ability to examine retrospective ontogenetic profiles. Sample size (i.e. weight of individual sample), however, may be limited due to the size of the vertebrae (related to the size of the species) and therefore the width of the growth rings that can be sampled. The extent to which elasmobranch vertebrae are mineralized, which is known to vary among species (Porter et al. 2006), will also affect the amount of untreated sample that is available for analysis, as the inorganic component contains less carbon per unit volume than the organic portion (Porter et al. 2006, Mateo et al. 2008, Newsome et al. 2010). Therefore, samples with more mineralization will require a higher weight of untreated sample to be analyzed. This, combined with the amount of sample available from each growth ring, may become a limiting factor for serial stable isotope analysis of vertebrae to provide reliable $\delta^{13}C$ and $\delta^{15}N$ values. Determining appropriate sample mass required for viable isotopic analysis of elasmobranch vertebrae is therefore warranted.

Typically samples for $\delta^{15}N$ analysis are untreated based on the assumption that inorganic N does not contribute to the total sample N (Brodie et al. 2011b). However, with the increased use of dual mode stable isotope analysis, which requires the input of only one sample to determine both $\delta^{13}C$ and $\delta^{15}N$ values, samples for both C and N analysis are commonly acid treated. Establishing if the $\delta^{15}N$ value of a sample is affected by EDTA treatment is therefore required, given that several studies have reported effects of HCl treatment on measured $\delta^{15}N$ values (Bunn et al. 1995, Jacob et al. 2005, Brodie et al. 2011b).

Considering the global archived collections of elasmobranch vertebrae for age and growth work, there is vast potential to undertake stable isotope analysis on these samples to reconstruct migration patterns and examine foraging behavior of sharks over ontogeny. This would allow analysis of current movement and feeding behaviors of species plus allow retrospective analysis of historical samples to examine human and/or potential climate mediated effects. Prior to their use an understanding of how acid treatment affects measured $\delta^{13}C$ and $\delta^{15}N$ of elasmobranch vertebral material is required. In this study, the effect of EDTA treatment on stable isotope values in elasmobranch vertebrae was investigated in three shark species: white (*Carcharodon carcharias*), tiger (*Galeocerdo cuvier*), and sand tiger (*Carcharias taurus*). Specifically, we examined (i) the minimum starting sample weight required for viable isotope data (EDTA treated and untreated), ii) the percentage sample yield following EDTA extraction, iii) the $\delta^{13}C$ values in the inorganic and organic portions of the vertebrae, and iv) the effect of EDTA treatment on $\delta^{13}C$ and $\delta^{15}N$ values.

EXPERIMENTAL

All samples were obtained from individuals that were incidentally caught in beach protection nets off KwaZulu-Natal, South Africa (for further details see Cliff and Dudley (2011)). Vertebrae were excised from five individuals of each species (white, tiger, and sand tiger sharks) anterior to the first dorsal fin during routine dissections by KwaZulu-Natal Sharks Board staff and stored frozen. Prior to analysis, samples were defrosted, cleaned of excess tissue and dried for 48 h at 40°C. Bow-tie sections were cut from each vertebral centrum using an IsoMet® low speed diamond saw (Beuhler Canada, Whitby, ON, Canada). The entire corpus calcareum was separated from the intermediala for each individual and ground into a fine powder. To create a homogenized mixture and remove any potential ontogenetic effects on measured stable isotope values the resulting powder was placed on an orbital shaker (VWR OS-500, VWR International, Mississauga, ON, Canada) for 8 h. The powder for each individual corpus calcareum was then split between two treatments: no treatment and EDTA treated.

EDTA treatment

To compare EDTA treated samples to untreated samples three replicate samples per individual (five individuals per species) weighing 3 mg each were taken from the homogeneous mixture and treated following the procedure outlined in Kim and Koch (2012). Briefly, sample powder was placed in a 2 mL cryovial and 1.5 mL of 0.5M EDTA (pH 8.0, Fisher Scientific) was added. Samples were vortexed for one minute. After allowing the reaction to proceed for one week at room temperature, samples were centrifuged for 15 min and the EDTA was removed, replaced, and the process was repeated. The samples were washed with Milli-Q water (Milli-Q RG, EMD Millipore

Corporation, Billerica, MA, USA) 10 times and sonicated for 10 min after the 1st, 5th, and 10th washes. Samples were soaked overnight after the 5th and 10th wash. Samples were then freeze-dried for 48 h and the remaining sample weighed into Sn capsules.

Minimal sample weight for isotopic analysis of elasmobranch vertebrae

Untreated samples were weighed in Sn capsules in triplicate for each individual shark in 200 μg increments from 200 μg to 1600 μg and analyzed for $\delta^{13}C$ and $\delta^{15}N$. The use of multiple consecutive weights of the homogenized untreated samples enabled an investigation of (i) the effect of sample weight on isotope values, and (ii) an appropriate minimum sample weight for viable isotope data, and also enabled (iii) a comparison of stable isotope data of untreated samples with those treated with EDTA.

To investigate the minimum sample weight required for EDTA treated vertebrae, 20 mg of the homogenized mixture from one individual per species was treated with EDTA. After EDTA treatment multiple consecutive weights increasing in mass from 200 μg to 1200 μg were weighed as described above. Minimum sample weight to obtain viable isotopic values for both EDTA treated and untreated vertebrae were determined as the point at which $\delta^{13}C$ and $\delta^{15}N$ values reached an asymptote.

Stable isotope analysis

All samples were analyzed with an elemental analyzer (Costech Instruments, Valencia, CA, USA) interfaced to a Thermo Finnigan Delta^{Plus} mass spectrometer (Thermo Finnigan, San Jose, CA, USA) at the Great Lakes Institute for Environmental Research, University of Windsor (Windsor, Canada). Stable isotope ratios are expressed in delta (δ) values as the ratio of an unknown sample to a recognized standard and are expressed in parts per thousand (or per mil, ‰) using the following equation:

$$\delta^{b}X = [(R_{sample}/R_{standard})-1] \times 1000 \quad (1)$$

where X is the element, b is the mass of the heavy isotope (less abundant) and R_{sample} and $R_{standard}$ are the heavy to light isotope ratio (for example; carbon, $^{13}C/^{12}C$, nitrogen: $^{15}N/^{14}N$) of the sample and standard, respectively (Peterson and Fry 1987). To measure analytical precision the standard deviation of a National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA) standard (NIST standard 8414-bovine liver) and an internal laboratory standard (fish muscle, tilapia (*Oreochromis niloticus*)) were used. The standard deviation was 0.2‰ for $\delta^{13}C$ (n=80) and 0.1‰ for $\delta^{15}N$ (n=80) for both standards. The accuracy of the NIST standards for $\delta^{13}C$ (NIST 8542 and NIST 8573) was within 0.17‰ and 0.09‰ of certified values, respectively, and for $\delta^{15}N$ (NIST 8547 and NIST 8549) was within 0.12‰ and 0.10‰, respectively.

Statistical analysis

Percentage sample yield, a proxy for the amount of collagen in the vertebrae, was calculated as the amount of vertebral material present after EDTA treatment compared to the amount of vertebral material before EDTA treatment, following Kim and Koch (2012). To determine the percent C (%C) in the inorganic and organic portions of the vertebrae and the $\delta^{13}C$ value of the inorganic C, a mass balance approach was adopted:

$$W*c_u = C_t \quad (2)$$

where W is the bulk sample weight, c_u is the total %C in the untreated sample (organic and inorganic) and C_t is the total amount of C. The amount of organic C in the sample was calculated as follows:

$$Y*W*c_e = C_o \quad (3)$$

where Y is equal to the percentage sample yield, c_e is the %C in the EDTA treated sample (organic) and C_o is the amount of organic C. To determine the amount of inorganic C we subtracted organic C from total C and converted these values to percentages. The $\delta^{13}C$ value of the inorganic portion of the vertebrae was calculated as follows:

$$z = \frac{T-(x*p)}{q} \quad (4)$$

where z is the $\delta^{13}C$ value for inorganic carbon, T is the $\delta^{13}C$ value of the untreated sample, x is the $\delta^{13}C$ value of the treated sample, p is the %C in the organic portion, and q is the %C in the inorganic portion.

Due to replicates and unequal sample sizes between untreated and EDTA treated vertebral samples, the effects of EDTA treatment on measured $\delta^{13}C$ and $\delta^{15}N$ values was examined using linear mixed effects models (LME). Three models were constructed and contrasted and the model with the lowest Akaike information criterion (AIC) was selected. The first model measured the variance in $\delta^{13}C$ and $\delta^{15}N$ values between individuals. The second model included EDTA treatment as a fixed effect to test between treatments among individuals, with all individuals assumed to be equally affected by EDTA treatment. The third model assumed random EDTA treatment effects (i.e., individuals respond differently to EDTA treatment). For all species and for both $\delta^{13}C$ and $\delta^{15}N$, the third model had the lowest AIC score in all cases and results for this are presented. For the LMEs, no evidence of an effect of EDTA treatment on measured $\delta^{13}C$ and $\delta^{15}N$ values in the samples was indicated by confidence intervals overlapping zero while evidence of an effect was indicated by confidence intervals that did not overlap zero. The strength of the effect is shown by the distance from zero; with a larger value

from zero indicating strong evidence for an effect and a value near zero indicating modest evidence of a small effect. The global statistic accounts for the unobserved portion of the population. The difference in $\delta^{13}C$ and $\delta^{15}N$ values (± standard error) between the EDTA treated and untreated vertebral samples for each species was calculated both for individual sharks and for the species overall. It was expected that EDTA treatment would decrease $\delta^{13}C$ values, a result of removing the inorganic mineralized component, but that $\delta^{15}N$ values would remain consistent given limited nitrogen in inorganic material. All statistical analyses were conducted using R version 2.15.1

RESULTS AND DISCUSSION

Minimum Sample Weight

Untreated samples had higher measured $\delta^{13}C$ values with increasing carbon amplitude until reaching an asymptote at an amplitude of ~1000 mV (Figure 2.1A). This value is in agreement with the recommendation of the isotope ratio mass spectrometer manufacturer to obtain consistent and reproducible results. The minimum untreated sample weight required to obtain a C amplitude of 1000 mV varied by species; ~800 μg for white shark, ~800 μg for tiger shark and ~1000 μg for sand tiger shark vertebrae (Figure 2.1B). The minimum sample weight to obtain an amplitude of 1000 mV for EDTA treated samples was lower for all species, reflecting the removal of the mineralized component; ~300 μg –white, ~300 μg –tiger, ~350 μg – sand tiger (Figure 2.1B).

In contrast to $\delta^{13}C$, the untreated samples had lower measured $\delta^{15}N$ values with increasing N amplitude, reaching an asymptote at an amplitude of ~1500 mV (Figure 2.2A), a value above the manufacturers recommendation of 1000mV. This trend was

observed in all individuals and for all three species (Figure 2.2A). To obtain a N amplitude of 1500 mV the minimum untreated sample weight was ~700 μg for white sharks and ~800 μg for tiger and sand tiger sharks (Figure 2.2B). For EDTA treated samples, ~250 μg of sample material was required for all three species (Figure 2.2B).

These results indicate measured elasmobranch vertebrae isotopic values may be artificially lower for $\delta^{13}C$ by ~ 0.6‰ (^{13}C; range 0.3-1.3) or higher for $\delta^{15}N$ by ~0.9‰ (^{15}N; range 0.1-1.3) when samples are below the minimum required weight. This is in agreement with previous work that reported unpredictable enrichments and depletions for low weight samples (Brodie et al. 2011a). These observed trends in measured stable isotope values associated with sample weight are a result of the total carbon and nitrogen levels in the sample material being below that of the instrument baseline, specific to the isotope ratio mass spectrometer used. Given that the erroneous isotope values occurred within the manufacturer recommended levels (i.e. 1000 mV for $\delta^{15}N$), it is necessary to report initial test runs of sample weight versus vertebrae isotope data, and to report accurate final sample weights analyzed.

Percentage Sample Yield

The percentage of vertebral material (i.e. collagen) present after EDTA treatment varied within and among species (Table 2.1). The percentage collagen yields following EDTA extraction were slightly below previously reported values (Kim and Koch 2012), but in agreement with quantitative collagen measures (Porter et al. 2006). Following Kim and Koch (2012), this demonstrates that EDTA is effective at removing the mineralized component within elasmobranch vertebrae. Due to the inter- and intra-species variability in percentage sample yield following EDTA treatment, a starting sample weight of 3 mg

of untreated material is recommended for EDTA extraction of all three species studied to obtain sufficient collagen weight for viable isotopic analysis.

On average the %C in the EDTA treated samples increased, due to the removal of inorganic carbon, relative to the untreated samples for each species; white shark %C increased from 16.6% ± 0.1 to 41.8% ± 0.1, tiger shark %C increased from 15.2% ± 0.05 to 42.0% ± 0.1, and sand tiger shark %C increased from 12.5% ± 0.1 to 41.4% ± 0.1. The level of mineralization in shark vertebrae is known to vary among species (Porter et al. 2006), such that shortfin mako (*Isurus oxyrinchus*) vertebrae have 39.2% dry mass mineralization, whereas gulper shark (*Centrophorus granulosus*) vertebrae have 55.1% dry mass mineralization (Porter et al. 2006). These differences in mineralization among species account for different material properties in the vertebrae required to accommodate different life strategies. As expected, the variability observed in the percentage sample yield data suggests good correspondence with the level of mineralization in the vertebrae, accepting that these structures also include proteoglycans and water. Following removal of the mineralized component by EDTA treatment, the %C in all three species was more similar and less variable, as expected. Sand tiger sharks had the lowest percentage sample yield of collagen indicating they had the highest level of mineralization and therefore a larger untreated sample weight was required for both untreated and EDTA extracted samples to obtain accurate isotope data (as evidenced above). Knowledge of the extent to which a species' vertebrae are mineralized will help predict the minimum untreated sample amount required.

Effects of EDTA treatment on $\delta^{13}C$ and $\delta^{15}N$ values

EDTA treatment had variable effects on measured $\delta^{13}C$ values in the vertebrae of

the three shark species examined relative to untreated samples (Table 2.2). White shark vertebrae treated with EDTA were enriched in ^{13}C relative to untreated samples (Figure 2.3A). The LME model found that EDTA treatment had an overall effect (Global statistic) on $\delta^{13}C$ values, however, at the individual level there was no treatment effect in one individual and the effect was minimal for two individuals. Tiger shark EDTA treated vertebral samples were both enriched and depleted in ^{13}C compared to untreated samples, indicating there was within species variation occurring (Figure 2.3A). The variation was within machine precision and therefore cannot be attributed to machine or EDTA induced error. For tiger sharks, the LME found no overall effect of EDTA treatment on the $\delta^{13}C$ values but there was a minor effect of treatment at the individual level (Table 2.2). Alternatively, sand tiger shark EDTA treated vertebral samples were all depleted in ^{13}C compared to the untreated samples (Figure 2.3A) and the LME model detected an effect of the EDTA treatment (both overall and at the individual level; Table 2.2). The enrichment of ^{13}C in EDTA treated vertebrae of white shark and some tiger shark samples compared to untreated samples was contradictory. It would be expected that vertebrae would be depleted in ^{13}C due to the removal of the ^{13}C rich inorganic portion (Mateo et al. 2008), potentially suggesting a biased caused by the EDTA treatment for these two species and/or the effect being less than machine precision.

The effects of EDTA as a pre-treatment agent for inorganic C removal prior to stable isotope measurements in elasmobranch vertebrae have not been investigated previously. However, significant effects on stable isotopic values have been documented for blood and muscle samples from quail (*Coturnix coturnix japonica*) (Hobson et al. 1997), blood from sheep (*Ovis aries*) (Hobson et al. 1997), and epidermis from turtles

(Barrow et al. 2008), that were preserved in dimethyl-sulfoxide (DMSO) containing EDTA. Previously, acid treatment using HCl has resulted in variable effects by species such that a significant depletion in ^{13}C values in invertebrates (Mateo et al. 2008) and algae and cyanobacteria (Ng et al. 2007) were reported, while no significant differences were found in molluscs (Ng et al. 2007), winter flounder (*Pleuronectes americanus*) or mud shrimp (*Crangon septemspinosa*) (Bosley and Wainright 1999). The variable effects of EDTA treatment on measured stable isotope values in this study mirror those reported for HCl treatment. The imprecision associated with the treatment method is typically not reported, however, if it is higher than instrument imprecision (which is normally reported) there could be implications for interpreting stable isotope values for ecological studies.

The percentage of inorganic and organic C in vertebrae from all three species was similar (Table 2.3), indicating that while sand tiger sharks had the highest amount of mineralization, the amount of inorganic C contained within the mineralized portion was similar to that of white and tiger sharks. The $\delta^{13}C$ values for the inorganic portion in white and tiger sharks were similar to those of the organic portion (Table 2.3), consistent with the small differences observed in $\delta^{13}C$ values between EDTA treated and untreated samples (~0.2‰ (Figure 2.4)). This value would not be considered ecologically significant for most applications, questioning the need for EDTA treatment in these two species. The sand tiger sharks, however, had a larger difference between the $\delta^{13}C$ values for organic and inorganic portions (Table 2.3) compared to white and tiger sharks, which may be a factor of differences in feeding strategies among species. The organic C in collagen is derived from the proteins of the individual's diet, while the inorganic C in the

mineralized portion is derived from the carbohydrates and lipids of an individual's diet (Krueger and Sullivan 1984). Therefore, the observed differences in $\delta^{13}C$ values between the organic and inorganic portions of the vertebrae may be driven by the individuals' metabolic pathways and/or growth requirements. There is potential for future research examining the differences observed between $\delta^{13}C$ values in the inorganic and organic portion of shark vertebrae to investigate trophic level effects on growth and energy pathways of a species.

When considering $\delta^{15}N$ values, the white shark EDTA treated vertebral samples were depleted in ^{15}N relative to untreated samples (Figure 2.3B). The LME model found an effect of EDTA treatment both overall and at the individual level in white shark vertebrae (Table 2.2). Tiger shark EDTA treated vertebral samples were both enriched and depleted in ^{15}N relative to untreated samples (Figure 2.3B), but there was no overall effect of EDTA treatment on $\delta^{15}N$. Treated sand tiger shark samples were depleted in ^{15}N relative to untreated samples (Figure 2.3B) and the LME model found there was both an overall and individual effect of EDTA treatment on $\delta^{15}N$ values (Table 2.2). The $\delta^{15}N$ values should not be affected by the removal of the inorganic component, as it is assumed that inorganic N does not contribute to the total N (Newsome et al. 2010, Brodie et al. 2011b). Brodie et al. (2011a, b) however, compared the effects of different acidification methods on $\delta^{15}N$ values in terrestrial and aquatic organisms and concluded that variability within and among methods/tissue analyzed complicates comparisons between species and studies. Additionally, depletion and enrichment in ^{15}N values following acidification of soil samples (Brodie et al. 2011b) and seagrass and shrimp (Bunn et al. 1995), respectively, have been observed.

In this study, EDTA treatment resulted in both the depletion (white, tiger, and sand tiger sharks) and enrichment (tiger sharks) of ^{15}N in shark vertebrae. Overall, the mean difference in $\delta^{15}N$ values between EDTA treated and untreated samples was less than 0.5‰ for all species (Figure 2.4), but the effect of EDTA treatment was variable among species and larger than the effect of EDTA on measured $\delta^{13}C$ values for the white and tiger shark (Figure 2.4). This would suggest EDTA extraction is not required for these species and may result in measured $\delta^{15}N$ values with larger uncertainties. Moreover, following EDTA extraction $\delta^{15}N$ values did not show a systematic enrichment or depletion indicating that the use of a correction factor is not suitable (i.e. the effect is non-linear, *sensu* Brodie et al. 2011b). For the sand tiger shark, it would appear EDTA extraction is required to correct $\delta^{13}C$ values, but the effect on $\delta^{15}N$ values must be acknowledged. Specifically, for higher trophic level species an increase in $\delta^{15}N$ of 1.2-1.8‰ between predator and prey has been described (Hussey et al. 2014), indicating that the unpredictable enrichments and depletions in ^{15}N caused by EDTA treatment may cause the incorrect trophic level to be assigned to a species. Additionally, several studies have used stable isotope analysis of multiple tissues (e.g. vibrissae, turtle scute, muscle, and blood) (Newsome et al. 2009, Vander Zanden et al. 2010, Matich et al. 2011) to investigate the degree of dietary specialization within a population. If this method were applied to elasmobranch vertebrae treated with EDTA the degree of specialization may be misinterpreted. Samples in this study were run using dual mode analysis, but to correct for the effect of EDTA treatment on $\delta^{15}N$ values of sand tiger vertebrae would require the analysis of paired samples per individual; one acid treated sample for C analysis and one untreated sample for N analysis, again assuming that inorganic N does not contribute to

total N. This approach may be restricted by the sample weight available (such as serially sampled elasmobranch vertebrae), as a larger sample will be required. Consequently, examining fine-scale ontogenetic changes in isotope values of growth bands may be limited for certain species, but coarse level profiles would be possible.

CONCLUSION

The unpredictable enrichment and depletion of ^{13}C and significant effect on $\delta^{15}N$ values in white, tiger, and sand tiger shark vertebrae following EDTA treatment raises questions over the suitability of this approach to remove inorganic C material from samples prior to bulk stable isotope analysis. Importantly, to obtain robust stable isotopic data from untreated and EDTA treated vertebral material required the analysis of species-specific weights of starting sample material. For the white and tiger shark, EDTA treatment resulted in minimal effects on $\delta^{13}C$ values, but had a more marked change on $\delta^{15}N$ values and is therefore not recommended. A correction factor is not suitable as the effect of EDTA treatment on measured $\delta^{13}C$ and $\delta^{15}N$ values is non-linear and varies both within and among species. For sand tiger sharks, there was a consistent effect of EDTA treatment on $\delta^{13}C$ values suggesting treatment is required, but its effects on $\delta^{15}N$ values must be accounted for. The differences in $\delta^{13}C$ values between the inorganic and organic portion of the vertebrae observed among species indicate there is potential to use these profiles to investigate metabolic pathways among species of different trophic levels and phylogeny.

When examining stable isotopes in elasmobranch vertebrae we recommend preliminary analysis of EDTA treated and untreated samples for the study species across different sample weights to identify (i) if EDTA treatment is required to obtain viable

$\delta^{13}C$ data and (ii) to determine the sample weight required (EDTA treated or untreated) for analytical precision. For studies where EDTA treatment is required for C analysis, untreated samples should be analyzed for N.

REFERENCES

Barrow LM, Bjorndal KA, Reich KJ (2008) Effects of preservation method on stable carbon and nitrogen isotope values. Physiol Biochem Zool 81: 688-693

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124: 483-494

Bosley KL, Wainright SC (1999) Effects of preservatives and acidification on the stable isotope ratios ($^{15}N:^{14}N$, $^{13}C:^{12}C$) of two species of marine animals. Can J Fish Aquat Sci 56: 2181-2185

Brodie CR, Leng MJ, Casford JSL, Kendrick CP, Lloyd JM et al. (2011a) Evidence for bias in C and N concentrations and $\delta^{13}C$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. Chem Geol 282: 67-83

Brodie CR, Heaton THE, Leng MJ, Kendrick CP, Casford JSL et al. (2011b) Evidence for bias in measured $\delta^{15}N$ values of terrestrial and aquatic organic materials due to pre-analysis acid treatment methods. Rapid Commun Mass Spectrom 25: 1089-1099

Bunn SE, Loneragan NR, Kempster MA (1995) Effects of acid washing on stable isotope ratios of C and N in Panaeid Shrimp and seagrass: implications for food-web studies using multiple stable isotopes. Limnol Oceanogr 40: 622-625

Cailliet GM, Goldman KJ (2004) Age determination and validation in chondrichthyan fishes. In: Carrier JC, Musick JA, Heithaus MR (eds) Biology of Sharks and their Relatives. CRC Press, New York, pp 399-447

Campana SE, Natanson LJ, Myklevoll S (2002) Bomb dating and age determination of large pelagic sharks. Can J Fish Aquat Sci 59: 450-455

Carabel S, Godínez-Domínguez E, Verísimo P, Fernández L, Freire J (2006) An assessment of sample processing methods for stable isotope analyses of marine food webs. J Exp Mar Biol Ecol 336: 254-261

Cliff G, Dudley SFJ (2011) Reducing the environmental impact of shark control programs: a case study from KwaZulu-Natal, South Africa. Mar Freshw Res 62: 700-709

Dean MN, Summers AP (2006) Mineralized cartilage in the skeleton of chondrichthyan fishes. Zoology 109: 164-168

DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495-506

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341-351

Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeing ecology in white sharks. Ecology 87: 829-834

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314-326

Hobson KA, Gibbs HL, Gloutney ML (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. Can J Zool 75: 1720-1723

Hussey NE, MacNeil MA, Olin JA, McMeans BC, Kinney MJ et al. (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol 80: 1449-1484

Hussey NE, MacNeil MA, McMeans BC, Olin JA, Dudley SFJ et al. (2014) Rescaling the trophic structure of marine food webs. Ecol Lett 17: 239-250
Jacob U, Mintenbeck K, Brey T, Knust R, Beyer K (2005) Stable isotope food web studies: a case for standardized sample treatment. Mar Ecol Prog Ser 287: 251-253
Johnson BJ, Fogel ML, Miller GH (1998) Stable isotopes in modern ostrich eggshell: A calibration for paleoenvironmental application in semi-arid regions of southern Africa. Geochim Cosmochim Acta 62: 2451-2461
Kerr LA, Andrews AH, Cailliet GM, Brown TA, Coale KH (2006) Investigations of $\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$ in vertebrae of white shark (*Carcharodon carcharias*) from the eastern North Pacific Ocean. Environ Biol Fish 77: 337-353
Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fish 95: 53-63
Krueger HW, Sullivan CH (1984) Models for carbon isotope fractionation between diet and bone in stable isotopes in nutrition. In: Turnland T, Johnson PE (eds) American Chemical Society, Symposium Series 258. Washington, DC pp 205-220
Larson TE, Heikoop JM, Perkins G, Chipera SJ, Hess MA (2008) Pretreatment technique for siderite removal for organic carbon isotope and C:N ratio analysis in geological samples. Rapid Commun Mass Spectrom 22: 865-872
Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88: 42-48
MacNeil MA, Skomal GB, Fisk AT (2005) Stable isotopes from multiple tissues reveal diet switching in sharks. Mar Ecol Prog Ser 302: 199-206
Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J Anim Ecol 80: 294-305
Mateo MA, Serrano O, Serrano L, Michener RH (2008) Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. Oecologia 157: 105-115
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48: 1135-1140
Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K et al. (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90: 961-974
Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Mar Mammal Sci 26: 509-572
Ng JSS, Wai TC, Williams GA (2007) The effects of acidification on the stable isotope signatures of marine algae and molluscs. Mar Chem 103: 97-102
Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320
Porter ME, Beltrán JL, Koob TJ, Summers AP (2006) Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondrichthyes). J Exp Biol 209: 2920-2928
R Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
Tuross N, Fogel ML, Hare PE (1988) Variability in the preservation of the isotopic

composition of collagen from fossil bone. Geochim Cosmochim Acta 52: 929-935
Vander Zanden HB, Bjorndal KA, Reich KJ, Bolten AB (2010) Individual specialists in a generalist population: results from a long-term stable isotope series. Biol Lett 6: 711-714

Table 2.1 Mean (± standard error (SE)) percentage sample yield after EDTA treatment of shark vertebrae in three species of sharks.

Species	Individual	n	Mean Percentage Sample Yield (± SE)	Species Mean Sample Percentage Yield (± SE)
white	1	3	16.9 ± 1.36	16.1 ± 1.24
	2	3	21.0 ± 2.43	
	3	3	17.1 ± 0.57	
	4	3	17.8 ± 0.84	
	5	3	7.0 ± 0.99	
tiger	1	3	12.4 ± 1.32	15.5 ± 0.78
	2	3	14.2 ± 2.07	
	3	3	14.3 ± 1.64	
	4	3	16.2 ± 1.54	
	5	3	18.4 ± 0.96	
sand tiger	1	3	10.8 ± 2.42	11.4 ± 1.15
	2	3	15.6 ± 0.42	
	3	3	10.8 ± 2.42	
	4	3	14.3 ± 2.90	
	5	3	7.2 ± 1.92	

Table 2.2 Results of linear mixed effects model for $\delta^{13}C$ and $\delta^{15}N$ values between EDTA treated and untreated vertebral samples from three species of sharks.

Sample	Estimate	95% Confidence Interval	Sample	Estimate	95% Confidence Interval
white shark $\delta^{13}C$			white shark $\delta^{15}N$		
Global	0.16	0.06, 0.27	Global	-0.39	-0.69, -0.09
1	0.28	0.18, 0.36	1	-0.91	-1.02, -0.80
2	0.14	0.05, 0.23	2	-0.22	-0.33, -0.11
3	0.10	0.01, 0.19	3	-0.33	-0.43, -0.22
4*	0.05	-0.04, 0.14	4*	-0.04	-0.15, 0.07
5	0.25	0.16, 0.34	5	-0.45	-0.56, -0.34
tiger shark $\delta^{13}C$			tiger shark $\delta^{15}N$		
Global*	-0.27	-1.01, 0.46	Global*	-0.27	-0.58, 0.05
1	-0.14	-0.27, -0.01	1	-0.70	-0.83, -0.57
2	0.30	0.18, 0.42	2	-0.27	-0.39, -0.14
3	-1.69	-1.82, -1.57	3	-0.34	-0.46, -0.21
4*	-0.08	-0.21, 0.04	4	-0.28	-0.41, -0.16
5	0.26	0.13, 0.38	5	0.26	0.13, 0.38
sand tiger shark $\delta^{13}C$			sand tiger shark $\delta^{15}N$		
Global	-0.54	-0.66, -0.43	Global	-0.44	-0.62, -0.27
1	-0.63	-0.69, -0.56	1	-0.38	-0.47, -0.28
2	-0.57	-0.64, -0.51	2	-0.27	-0.37, -0.17
3	-0.49	-0.55, -0.42	3	-0.74	-0.83, -0.64
4	-0.50	-0.56, -0.43	4	-0.47	-0.57, -0.38
5	-0.53	-0.59, -0.46	5	-0.36	-0.45, -0.26

* indicates there is no evidence of an effect of the EDTA treatment on the sample

Table 2.3 Measured $\delta^{13}C$ values (± SE) for the total and organic portions and calculated $\delta^{13}C$ (± SE) for the inorganic portion in vertebrae from three species of sharks. The calculated percentage (± SE) organic and inorganic C is also presented.

Species	Untreated $\delta^{13}C$ (‰)	Organic $\delta^{13}C$ (‰)	Inorganic $\delta^{13}C$ (‰)	Difference between Organic and Inorganic $\delta^{13}C$ (‰)	Organic C (%)	Inorganic C (%)
white	-11.9 ± 0.16	-11.7 ± 0.14	-12.0 ± 0.19	0.3	40.6 ± 6.2	59.4 ± 6.2
tiger	-12.0 ± 0.33	-12.2 ± 0.26	-11.9 ± 0.39	-0.3	38.6 ± 1.6	61.4 ± 1.6
sand tiger	-11.0 ± 0.17	-11.6 ± 0.15	-10.7 ± 0.15	-0.9	40.3 ± 4.3	59.7 ± 4.3

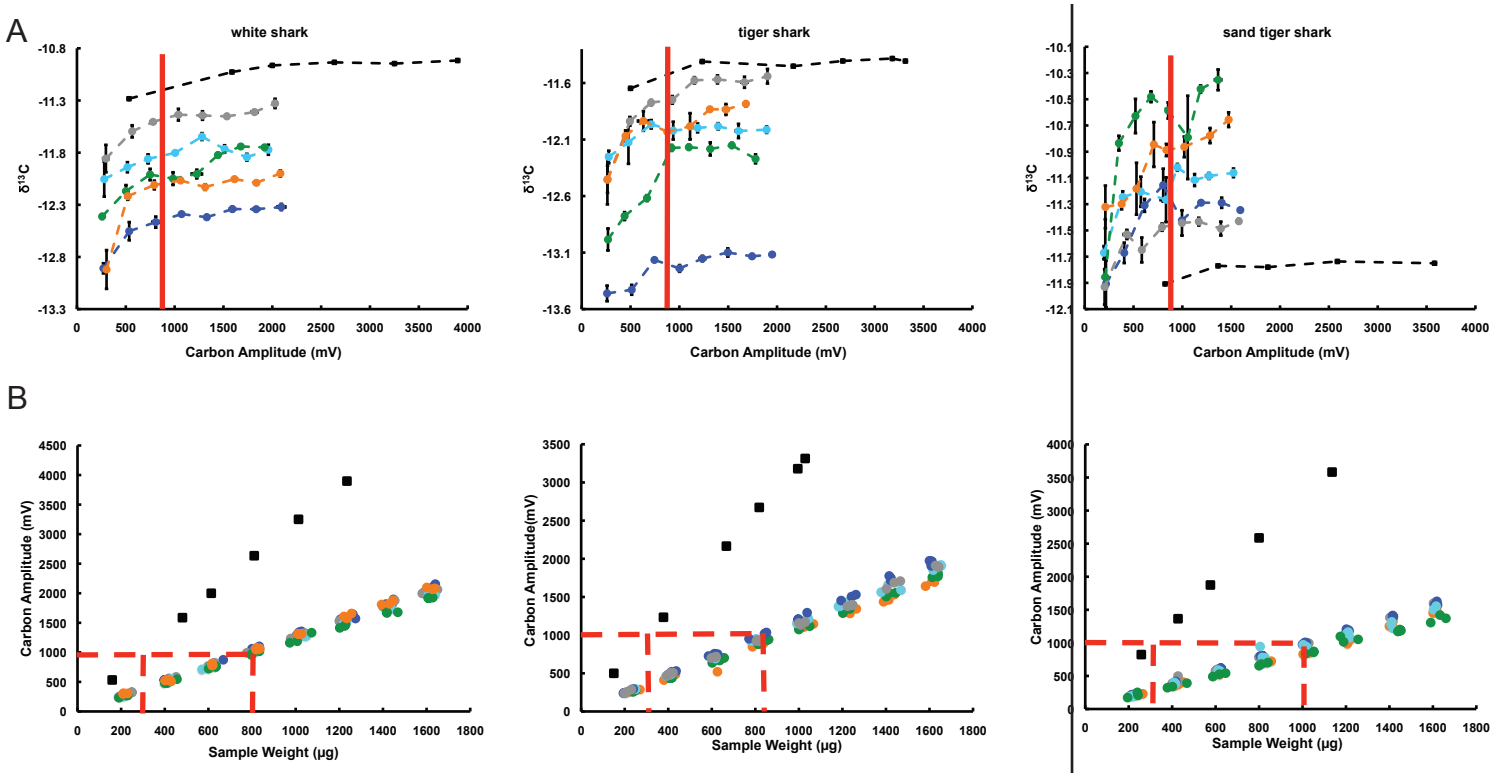

Figure 2.1 A- $\delta^{13}C$ values (±SE) of untreated and EDTA treated vertebral samples versus isotope ratio mass spectrometer carbon amplitude from three species of sharks. Samples from individual sharks are represented by a different color. Black squares represent the EDTA treated sample. Red vertical line indicates where values reach an asymptote. **B**- isotope ratio mass spectrometer carbon amplitude versus sample weight analyzed for untreated and EDTA treated vertebral samples from three species of sharks. Samples from individual sharks are represented by a different color. Black squares indicate EDTA treated sample. Red dashed lines indicate minimal weight required to obtain a carbon amplitude of 1000 mV.

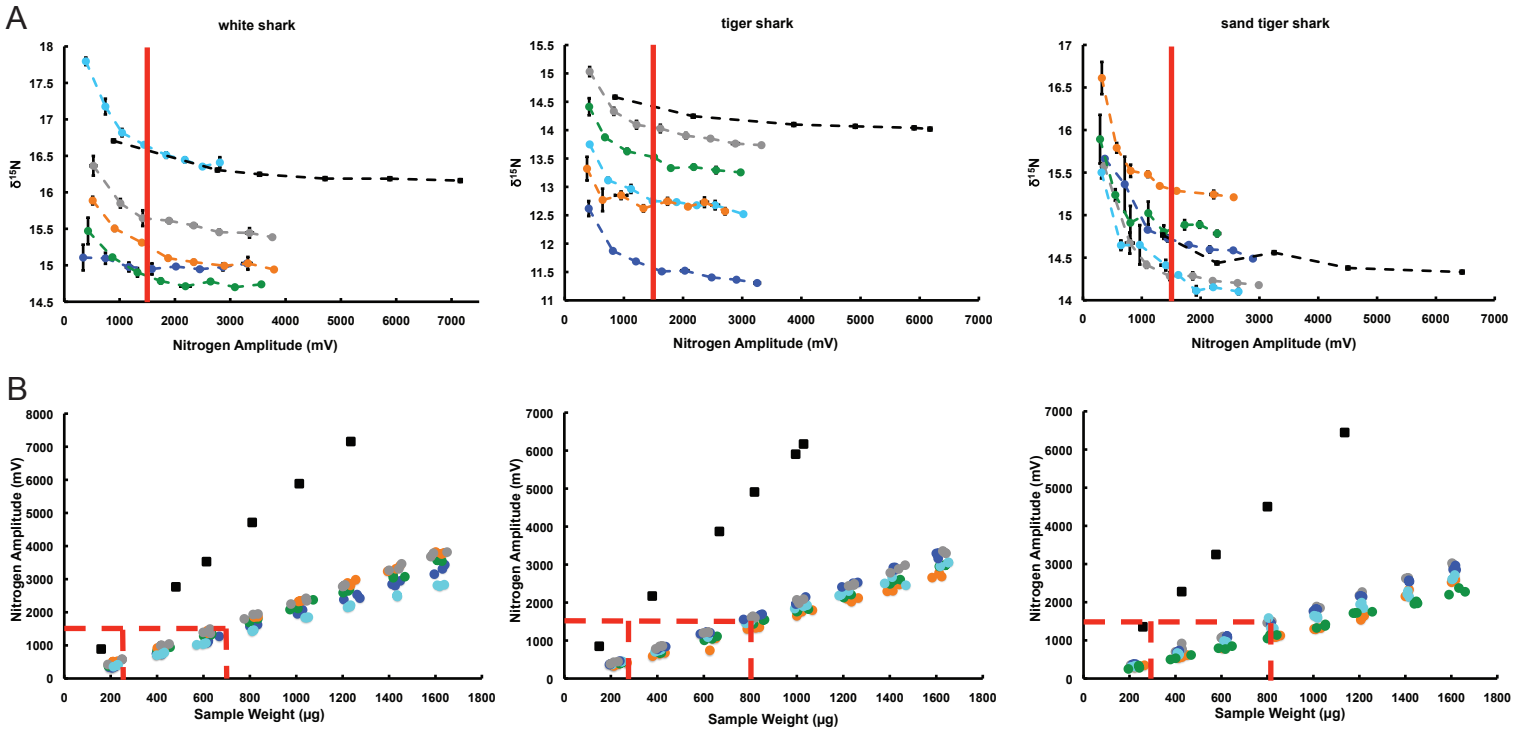

Figure 2.2 A- $\delta^{15}N$ values (±SE) of untreated and EDTA treated vertebral samples versus isotope ratio mass spectrometer nitrogen amplitude from three species of sharks. Samples from individual sharks are represented by a different color. Black squares represent the EDTA treated sample. Red vertical line indicates where values reach an asymptote. **B**- Isotope ratio mass spectrometer nitrogen amplitude versus sample weight analyzed for untreated and EDTA treated vertebral samples from three species of sharks. Samples from individual sharks are represented by a different color. Black squares indicate EDTA treated sample. Red dashed lines indicate minimal weight required to obtain a nitrogen amplitude of 1500 mV.

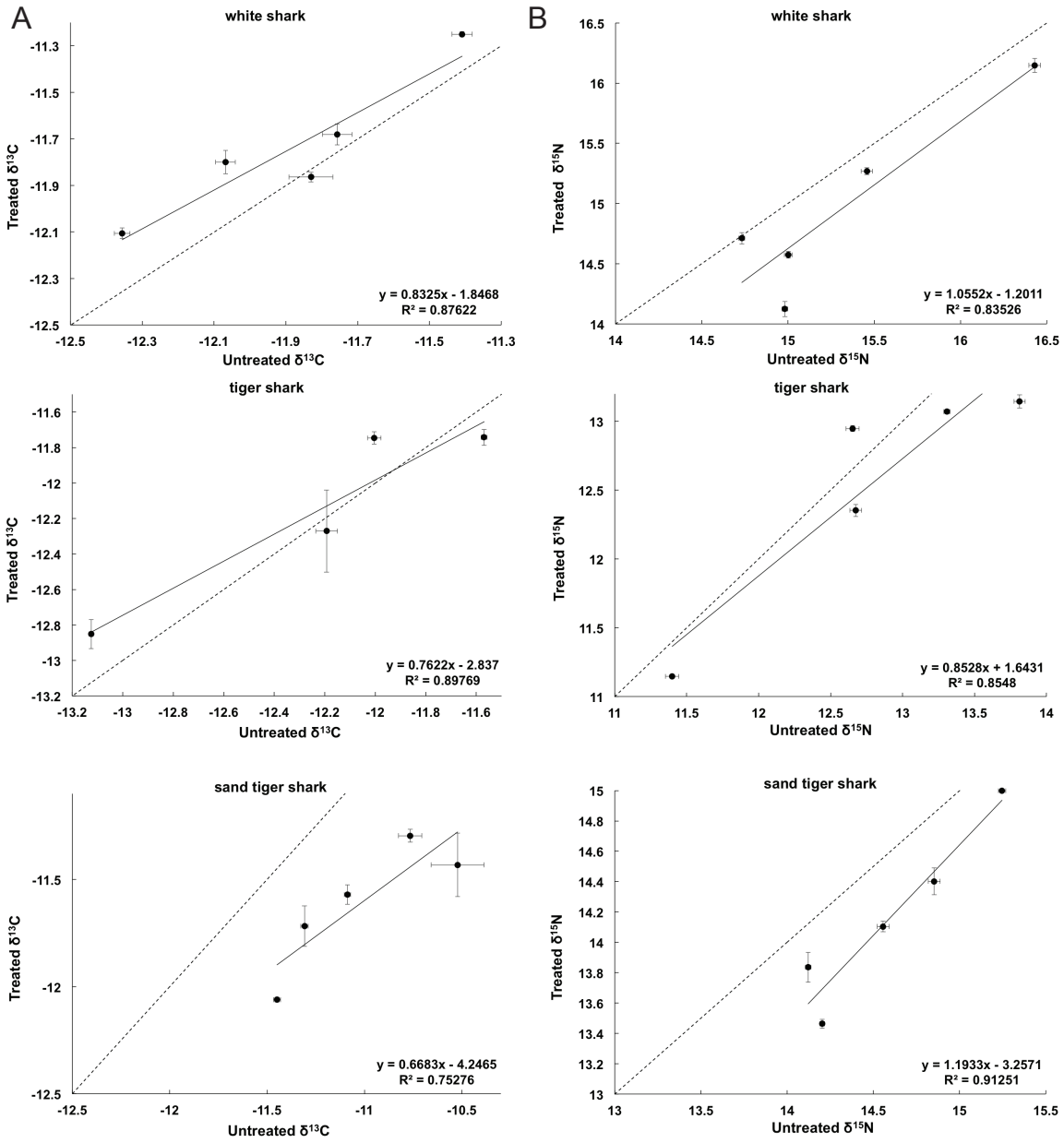

Figure 2.3 Stable isotope values (±SE) of EDTA treated versus untreated vertebral samples for white, tiger and sand tiger sharks: **A**- $\delta^{13}C$; **B**- $\delta^{15}N$. Black solid line is linear regression of isotope values between treatments. Black dashed line indicates the one to one relationship.

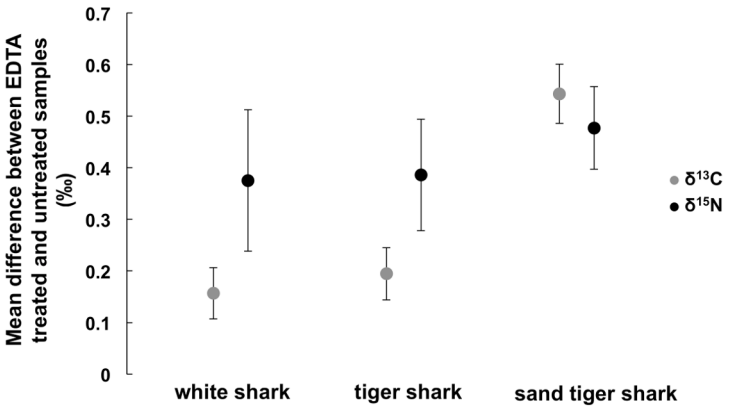

Figure 2.4 Mean difference (± SE) in measured stable isotope values between EDTA treated and untreated vertebral samples for white, tiger and sand tiger sharks. Gray circles indicate difference for $\delta^{13}C$ values and black circles indicate difference for $\delta^{15}N$ values.

CHAPTER 3: USING BOMB RADIOCARBON TO ESTIMATE AGE AND GROWTH OF THE WHITE SHARK, *CARCHARODON CARCHARIAS*, FROM THE SOUTHWESTERN INDIAN OCEAN[2]

INTRODUCTION

Conservation of threatened species is complicated by data deficiencies such as accurate age estimates. Age estimates are central to the determination of a species' growth rate, longevity, and age at sexual maturity; parameters that enable more effective management planning (Campana 2001, Cailliet and Goldman 2004, Cailliet and Andrews 2008). In the past the inclusion of inaccurate age estimates in stock assessments of several commercially important marine species has led to overexploitation of stocks (Lai and Gunderson 1987, Campana 2001, Cailliet and Andrews 2008).

For elasmobranchs, the majority of age and growth studies have relied on calcified structures, usually the vertebral centra, to estimate the age of an individual (Ridewood 1921, Cailliet et al. 2006, Goldman et al. 2012). This ageing technique is based on the assumption that band deposition occurs with a fixed periodicity, typically on an annual basis, and that band pairs (one opaque and one translucent band that together represent one year) are counted to obtain an age estimate for each individual (Cailliet and Goldman 2004). However, the interpretation of annual band pairs can be complicated by the presence of non-annual growth marks and other factors such as somatic growth that have been found to influence band deposition, e.g., Pacific angel shark (*Squatina californica*, Natanson and Cailliet 1990), basking shark *(Cetorhinus maximus*, Natanson et al. 2008), and wobbegong sharks (*Orectolobidae ornatus, O. maculatus, and O. halei*,

[2] Christiansen HM, Campana SE, Fisk AT, Cliff G, Wintner SP, Dudley SFJ, Kerr LA, Hussey NE. Using bomb radiocarbon to estimate age and growth of the white shark, *Carcharodon carcharias*, from the southwestern Indian Ocean. Mar Bio Manuscript number MABI-D-16-00016

Huveneers et al. 2013). Annual periodicity of band pairs must consequently be validated for each species and across the size range of animals for a species (Beamish and McFarlane 1983, Cailliet et al. 2006).

Bomb radiocarbon (^{14}C) dating has become a valuable tool to validate annual band pair formation in elasmobranchs (Goldman et al. 2012). This method capitalizes on the rapid increase of radiocarbon in the world's oceans as a result of atmospheric testing of thermonuclear devices in the 1950s and 1960s (Druffel and Linick 1978, Broecker and Peng 1982). The synchronous uptake of radiocarbon acts as a time stamp for year of formation in accretionary structures, e.g., otoliths (Kalish 1993, Campana 1999), molluscs (Weidman and Jones 1993), and elasmobranch vertebrae (Campana et al 2002). As a result, the $\Delta^{14}C$ value of consecutive samples from such tissues can be compared to a reference chronology to determine the year of formation for each band in the tissue sampled. Following this approach, bomb radiocarbon analysis has confirmed annual band pair formation in the vertebrae of several shark species including juvenile to adult animals (Campana et al. 2002, Ardizzone et al. 2006, Kneebone et al. 2008, Passerotti et al. 2010). It has also revealed, however, that vertebral growth bands may not accurately reflect the correct number of years after a certain stage/ time in an individual's lifetime. This phenomenon has been reported in sandbar (*Carcharhinus plumbeus*, Andrews et al. 2011), sand tiger (*Carcharias taurus*, Passerotti et al. 2014), and white (*Carcharodon carcharias*, Hamady et al. 2014, Andrews and Kerr 2015) sharks, and is commonly referred to as 'missing time' (Passerotti et al. 2014). Moreover, bomb radiocarbon analysis has also found that annual band pair formation can vary between populations of the same species; for example, annual band pair formation was validated to 26 years for porbeagle

sharks (*Lamna nasus*) in the northwest Atlantic Ocean (NWA, Campana et al. 2002), while in New Zealand annual band pair deposition was confirmed to approximately 20 years, but ages had been underestimated by up to 20 years in individuals older than 20 (Francis et al. 2007). This indicated vertebral growth had slowed or ceased in the slower-growing New Zealand population (Francis et al. 2007). Presumably the same phenomenon is responsible for the missing band pairs in the other shark studies (Natanson et al. 2014, 2015).

The white shark is a globally distributed, large marine predator that is listed as vulnerable on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and protected throughout its range by inclusion on Appendices I and II of the Convention on Migratory Species (CMS). International trade of white sharks is further regulated by listing on Appendix II of the Convention on International Trade of Endangered Species of Flora and Fauna (CITES). Age-reading studies of white sharks in the northeast Pacific Ocean (NEP, Cailliet et al. 1985), the southwest Indian Ocean (SWI, Wintner and Cliff 1999), and off the coast of Japan (Tanaka et al. 2011) suggested a maximum age of 12 to 15 years. These estimates were obtained by analyzing x-radiographs (x-rays) of vertebral centra and assumed annual deposition of band pairs. Initial attempts to validate annual periodicity of band pair counts in white sharks through the use of oxytetracycline in the SWI (Wintner and Cliff 1999) and bomb radiocarbon analysis for the NEP (Kerr et al. 2006), were inconclusive. Recently, Hamady et al. (2014) confirmed annual band pair formation for the NWA population up to 44 years with a suggested longevity of ~73 years, when accounting for a slowing or cessation of band pair deposition in the outer portion of the vertebral centra. Following this study,

Natanson and Skomal (2015) determined age and growth parameters for a larger number of individuals in the NWA, finding that white sharks can live to a minimum of 44 years and mature much later than previously estimated (26 and 33 years for males and females, respectively). Following reanalysis of bomb radiocarbon data for white sharks in the NEP and accounting for slowing or ceased growth in the vertebral centrum, Andrews and Kerr (2015) suggested that the white sharks in their study lived for > 30 years.

The objective of the current study was to validate annual band pair deposition in the vertebrae of white sharks from the SWI using bomb radiocarbon dating and to estimate standard growth parameters from band pair counts for a larger number of individuals. In addition, a subset of specimens from the NWA and NEP were also processed following our methods to allow a comparison of bomb radiocarbon data among regions and to illustrate methodological differences.

METHODS

A total of 55 white shark vertebrae were obtained from the KwaZulu-Natal Sharks Board (KZNSB), KwaZulu-Natal, South Africa. The KZNSB maintains a series of bather protection nets and drumlines along the coastline of KwaZulu-Natal on the east coast of South Africa (Cliff and Dudley 2011). Captured live specimens are tagged and released according to KZNSB protocols, while deceased individuals in good condition are transported to the laboratory for biological sampling. Fifty-three individual white sharks were sampled from 1991-2009, comprising 28 females (140-422 cm fork length (FL)) and 25 males (151-414 cm FL). Additional vertebral centra were obtained from an individual captured off Gansbaai on South Africa's Western Cape coast in 1987 (female, 537 cm FL) and from an individual captured off the coast of Malindi, Kenya, in 1996

(female, 487 cm FL). For all sharks included in this study sex, maturity, and precaudal length (PCL) were recorded. PCL was measured as the straight-line distance from the tip of the snout to the precaudal notch as defined by Dudley et al. (2005). All lengths were converted to straight fork length using the following equation: FL=1.100 PCL + 3.554 (n=142; Cliff et al. 1996a).

For both bomb radiocarbon analysis and band pair counts, vertebral centra were excised anterior to the first dorsal fin and stored frozen. Prior to analysis, vertebrae were cleansed of excess tissue and oven dried for 48 hrs at 40°C. A single vertebral section from each individual was prepared by a single cut using paired blades separated by a spacer on an IsoMet® low speed diamond saw (Buehler-Whitby, ON, Canada) to produce a bow-tie section 1-mm thick.

For age-reading (band pair counts), sections were digitally photographed (Spot Insight camera, Sterling Heights, MI, USA) at 2048 x 2048 resolution under a binocular microscope (Wild M5, Leica Microsystems, ON, Canada) at 16-40X magnification using reflected light while immersed in ethanol. Age interpretation was based on images enhanced for clarity and contrast using Adobe Photoshop CS2 using image enhancement methods described by Campana (2014) and using the band interpretation criteria of Natanson et al. (2002). Three non-consecutive counts of translucent bands visible in the corpus calcareum (Figure 3.1) were made by a single reader (SE Campana) without knowledge of shark length or any previous age estimate.

Samples for bomb radiocarbon assays were extracted from growth bands visible in the corpus calcareum from each of four selected SWI white sharks (Table 3.1), while working at 16X magnification under a binocular microscope. Extracted samples were

isolated as solid pieces using a Gesswein high-speed hand tool fitted with steel bits <1 mm in diameter. Each of two sharks had one sample extracted from an area near the birth band of the vertebra (Table 3.2), while each of two sharks had three samples extracted from various positions along the growth sequence of the corpus calcareum (Table 3.2; n=8 extracted samples total). The presumed date of band formation was back calculated from the band pair age and year of shark collection. After sonification in Super Q water and drying, extracted samples were weighed to the nearest 0.1 mg in preparation for ^{14}C assay with accelerator mass spectrometry (AMS). AMS assays also provided $\delta^{13}C$ (‰) values, which were used to correct for isotopic fractionation effects. Radiocarbon values were subsequently reported as $\Delta^{14}C$, which is the per mille (‰) deviation of the sample from the radiocarbon concentration of 19th-century wood, corrected for sample decay prior to 1950 according to methods outlined by Stuiver and Polach (1977). The mean standard deviation of the individual radiocarbon assays was ~5‰.

To enable a comparison among samples from this study and recently published $\Delta^{14}C$ values for white sharks in other regions, a subsample of white sharks from the NEP (n=3; Kerr et al. 2006) and the NWA (n=3; Hamady et al. 2014) were re-assayed. The analytical protocol for the preparation, extraction, and ^{14}C analysis of these samples was identical to that described above for the SWI animals, i.e. demineralization was not undertaken and all samples were taken from the corpus calcareum (Table 3.1). Independent of previously published age estimates for these sharks, ages were determined by counting band pairs as detailed above. Samples for bomb radiocarbon analysis were extracted from the first growth band pair after the birth mark for all three white sharks from the NEP (Table 3.2). For one individual (WH 17) an additional two assay samples

were extracted along the corpus calcareum closer to the outer edge from approximately the same locations as the original study (Kerr et al. 2006). Assay samples from the NWA sharks were removed from the first formed band pair after the birth mark for one individual (WS 100). For WS 57 and WS 105 two samples each were extracted along the corpus calcareum (Table 3.2).

To assign a date of formation to each bomb radiocarbon assay sample, it is necessary to compare the $\Delta^{14}C$ value of the unknown sample to the $\Delta^{14}C$ value of an environmentally similar reference chronology. In the absence of a local $\Delta^{14}C$ reference chronology, two substitute reference chronologies were used for all three white shark populations. First, we compared white shark $\Delta^{14}C$ values with a previously validated chronology from a hermatypic coral (*Porites lutea*) from Watamu Reef, Kenya (Grumet et al. 2002, Passerotti et al. 2014). It was assumed that the Watamu Reef coral $\Delta^{14}C$ chronology would be similar to the SWI region because both regions have a common source of oceanic water (South Equatorial Current of the Indian Ocean; Gordon 1986, Passerotti et al. 2014). Due to the documented phase lag of $\Delta^{14}C$ values in shark species, the white shark $\Delta^{14}C$ values were also compared with a validated chronology for the porbeagle shark from the NWA (Campana et al. 2002), which has been used as an indicator of how ^{14}C depleted carbon sources may be reflected in shark species. When plotting white shark $\Delta^{14}C$ values against the reference chronologies, annual band pair deposition was assumed and $\Delta^{14}C$ values were plotted at year of band formation for each assay sample. To provide an estimate of age range for each individual assay sample from the SWI, i.e. a measure of potential age error, the difference between collection year and year of band pair formation was multiplied by the coefficient of variation (CV; Campana

2001, Ardizzone et al. 2006), which was calculated for the combined set (bomb radiocarbon and age estimation) of white shark ages from the SWI (n=55). If the assigned year of formation was to the left of the reference chronology it is possible the age was overestimated, while a shift to the right of the reference chronology indicates possible age underestimation. To estimate the potential range in ages for each individual for the SWI samples, the year of formation for the earliest sample assayed was shifted to align with the Watamu reef coral chronology (maximum) or the porbeagle reference chronology (minimum).

To determine ages for the larger sample size of sharks from the SWI (n=51, excluding individuals analyzed for bomb radiocarbon), band pairs were counted as described above. To examine the variation of within reader counts, the coefficient of variation (Campana 2001) was calculated. The first clear growth band following the birth mark was presumed to represent the first year of growth (Casey et al. 1985). An angle change in the corpus calcareum was also used to confirm the identity of the birth mark, if present. To confirm the location of the birth mark, measurements from the midpoint of the isthmus to the birth mark were made, the relationship between vertebral radius (VR) and FL was calculated, and size at birth was back calculated using a modified Dahl-Lea method (Cailliet and Goldman 2004).

To estimate growth parameters for white sharks from the SWI the Schnute (1981) growth model was fit to length-at-age estimates based on observed band pair counts for the 55 individuals. Due to low sample sizes, sexes were combined for all analyses. The Schnute general model is as follows:

$$L_t = \left[L_1^b + \left(L_2^b - L_1^b\right)\frac{1-e^{-a(t-t_1)}}{1-e^{-a(t_2-t_1)}}\right]^{\frac{1}{b}},$$

where L_t is the length at time t (in years), L_1 is the length at age t_1, L_2 is the length at age t_2, t_1 is set to the lowest observed age (t_1=1), t_2 is set to the highest observed age (t_2= 38), a is a constant (time $^{-1}$) describing the constant relative rate of the relative growth rate, b is a dimensionless constant describing the incremental relative rate of the relative growth rate, where $a \neq 0$ and $b \neq 0$. To fit several of the more commonly used growth models the values of parameters a and b were adjusted; for the von Bertalanffy model $a > 0$ and $b = 1$ and for the logistic growth model $a > 0$ and $b = -1$ (Schnute 1981). The Gompertz function was described by the following (where $a > 0$ and $b = 0$; Schnute 1981):

$$L_t = L_1 e^{ln\left(\frac{L_2}{L_1}\right)\frac{1-e^{-a(t-t_1)}}{1-e^{-a(t_2-t_1)}}}$$

Estimates for each parameter (a, b, L_1, L_2) were determined using nonlinear least-squares regression methods in R (R Core Team 2014). Common parameter estimates (e.g. asymptotic size, L_∞, and theoretical size at birth, t_0) were calculated following Schnute (1981). Length at birth (L_0) was estimated from the resulting equation for each growth model. Using the "nlstools" package in R, 95% confidence intervals (CI) were constructed for parameter estimates via bootstrap methods (Baty and Delignette-Muller 2011). Final model selection was determined by lowest AIC_c value (Akaike 1973, Burnham and Anderson 2002) and concordance with known biological parameters. The AIC_c difference (Δ_i) of each model was calculated based on the lowest observed AIC_c value ($AIC_{c,\ min}$) as $\Delta_i = AIC_{c,\ i} - AIC_{c,\ min}$) to provide an estimate of the magnitude of difference between each model and the best model in the set. The model with the lowest AIC_c value was considered to have strong support and models with values of $\Delta_i < 2$ were considered indistinguishable in terms of fit (Burnham and Anderson 2002). In the case of models having values of $\Delta_i < 2$, known biological parameters were used to determine the

best fit model. To approximate model likelihood, the Akaike weight (w_i) of each model was also calculated (Burnham and Anderson 2002). The growth model analyses were then repeated using ages adjusted to align with the reference chronologies (Watamu Reef coral and porbeagle, separately) for the four specimens that underwent bomb radiocarbon analysis.

RESULTS

Bomb radiocarbon analysis

Eight samples from four SWI sharks were analyzed for $\Delta^{14}C$ (Table 3.2); ages based on band pair counts were estimated as 18-38 years. Assay samples with the earliest date of formation for the SWI sharks, 1955.5 and 1957.5, were relatively enriched in ^{14}C (-0.6 ‰ TRA92004 and -8.0 ‰ Gansbaai, respectively; Figure 3.2) and no samples were available to measure pre-rise $\Delta^{14}C$ values for white sharks from this region. The $\Delta^{14}C$ values of samples from TRA92004 and the first two assay samples from Gansbaai were to the left of the coral chronology. When accounting for analytical error and ageing error (CV) these values overlapped with the coral chronology and values for the remaining two sharks (Kenya96 and IFA91016) aligned with the porbeagle chronology (Figure 3.2). The error associated with assigned year of band pair formation for each sample (CV) ranged between 0.6 and 6.4 years (Table 3.2). Accepting the above errors, the observed sample proximity to the reference chronologies provides support for annual band pair formation for white sharks in the SWI up to 38 years. Shifting the year of sample formation to align with the coral reference chronology altered ages by -5.5 years to 11 years; while, aligning assay samples with the porbeagle reference chronology altered ages by -10.5 to 4 years (Table S3.1).

The range of values for the NWA (-77.1 to 10.9‰) and the NEP (-181.2 to -3.7‰) sharks captured pre-bomb values and the rapid increase of $\Delta^{14}C$ (Table 3.2). The $\Delta^{14}C$ values for the NWA specimens were within the range of values reported by Hamady et al. (2014; Table 3.3), while samples from the NEP specimens had more depleted $\Delta^{14}C$ values than those reported by Kerr et al. (2006; Table 3.3). Samples from two specimens (WH 1 and WS 100) aligned with the coral reference chronology (Figure 3.2). For three specimens (WH 17, WS 57, and WS 105) the timing of rapid increase in $\Delta^{14}C$ aligned with the porbeagle reference chronology (Figure 3.2). For one specimen (WH 3) the single assay sample was more depleted than both reference chronologies, therefore we were unable to determine if it aligned with a reference chronology (Figure 3.2).

Age determination

The FL-VR relationship was best described by a linear equation (Figure 3.3). The mean birth mark (± standard deviation) measurement was 8.6 ± 0.16 mm (n=36) from the isthmus of the vertebrae. The modified Dahl-Lea back calculated size at birth was 127 ± 2.0 cm FL. This back calculated birth size agrees with the previous values of 100 cm PCL (back calculated) and 135 cm PCL (predicted value) for white sharks from South Africa (Wintner and Cliff 1999) and is within the range of size at birth reported for other white shark populations (120-150 cm total length; Francis 1996, Uchida et al. 1996, Natanson and Skomal 2015); indicating the birth mark was correctly identified.

The youngest ages determined by band pair counts for the SWI were one (140 cm FL female) and four years old (151 cm FL male). The oldest estimated age for both sexes was 38 years (403 cm FL and 414 cm FL, female and male, respectively; Figure 3.4). The

CV for three replicate counts was 17.4%. Two mature females included in this study were aged 30 (487 cm FL) and 31 (537 cm FL) years; however, one 38-year-old female (403 cm FL) was immature. The three mature males were aged 16 (312 cm FL), 18 (352 cm FL), and 38 (414 cm FL) years.

Growth analysis

Growth curves were generated using band pair counts from the 55 vertebral samples including the four unadjusted ages from bomb carbon sampled animals (Figure 3.4). The Gompertz, logistic, and von Bertalanffy growth models all had Δ_i <2, indicating they were statistically indistinguishable (Table 3.4). The Gompertz growth curve had the lowest AIC value and estimated parameters that were the most biologically realistic; therefore it was chosen as the most appropriate model (Figure 3.4, Table 3.5). Using adjusted ages for the four bomb radiocarbon specimens to the porbeagle reference chronology along with the 51 ages estimated from band counts, the Schnute general model would not converge however, the other three models were statistically indistinguishable (Table S3.2). When the age for the four bomb radiocarbon specimens was adjusted to the coral reference chronology the Gompertz, logistic, and von Bertalanffy growth models all had Δ_i <2, indicating they were statistically indistinguishable. For all 7 models using adjusted age data, the AIC values were higher than when the original bomb radiocarbon ages were used. In addition, the confidence intervals for estimated parameters were greater for models that included adjusted ages (Table S3.3). Ages that were not adjusted to reference chronologies were consequently used as the best estimate for growth parameters of white sharks in the SWI (Figure 3.4; Table 3.5).

DISCUSSION

The current study provides evidence to support annual band deposition for white sharks from the SWI up to 38 years of age. The year of formation for assay samples of SWI white sharks were not adjusted to account for a cessation of band pair growth because individual error associated with the assigned year of formation (CV) overlapped with the $\Delta^{14}C$ reference chronologies. While the current precedent in the literature is to adjust ages to a reference chronology, there is no standard agreement on the most appropriate reference chronology to use (i.e. coral reference chronology versus porbeagle reference chronology; see Hamady et al. (2014), Andrews and Kerr (2015)). It is possible that the oldest sharks from the SWI in this study were over aged, however when data from this study were shifted to align with either reference chronology, growth model fit was worse and confidence intervals for model parameters were greater than for models with unadjusted data. Therefore, using the unadjusted ages was deemed to provide the best estimate of age and growth for white sharks in the SWI.

Recent studies using bomb radiocarbon to validate the age of white sharks in the NEP and NWA described years of life that were not recorded in the band pairs of the vertebrae, requiring the $\Delta^{14}C$ chronologies to be shifted to align with the reference chronology. Hamady et al. (2014) found that one individual estimated to have 44 band pairs corresponded with the bomb radiocarbon reference chronology, however, two individuals with 33 and 35 band pairs required age estimates to be corrected (+7 and -3, respectively) to align with the bomb radiocarbon reference chronology. In contrast, in the reanalysis of the Kerr et al. (2006) data, individuals previously aged as young as three years old required a correction for band pairs that were not continuously formed to align

with the reference chronology (Andrews and Kerr 2015). This correction increased the age estimate of three individuals by 9-15 years. The maximum age of these white sharks were limited by the coral reference chronology however, details on the early part of growth were not available for WH 17 and therefore the maximum age may have been higher (Andrews and Kerr 2015). The variability in occurrence of missing annuli among the three white shark studies indicates that the age at which growth ceases or slows is not consistent. Additional samples from pre-bomb years and during the rapid increase in $\Delta^{14}C$ would be required to fully determine if band pair deposition ceases in the vertebrae of the SWI population.

Diet is the main source of carbon in the vertebral collagen of sharks (Fry 1988, Campana et al. 2002) and the transfer of carbon from prey is considered to be slower than the direct uptake of dissolved inorganic carbon DIC (i.e., the carbon source for corals and fish otoliths). This delay can cause a phase shift in the $\Delta^{14}C$ values of vertebrae towards more recent years. Furthermore, this effect increases with trophic level and, for species that are known to feed on carbon-depleted sources (i.e., deep water prey), the effect is more evident (Campana et al. 2002, Kerr et al. 2006). White sharks off southern Africa use offshore (deep-water) resources (Hussey et al. 2012, Smale and Cliff 2012) that incorporate a more depleted $\Delta^{14}C$ value (Broecker and Peng 1982, Campana 1999). Thus, the slight lag of white shark $\Delta^{14}C$ values for two of the white sharks (IFA91016 and Kenya96) compared to the Watamu Reef coral reference chronology is expected, and has been observed in several species including the porbeagle (Campana et al. 2002) and shortfin mako (*Isurus oxyrinchus*, Ardizzone et al. 2006) sharks. Accepting age and analytical error, the Gansbaai assay samples approximately aligned with the Watamu

Reef coral chronology then shifted to approximately align with the porbeagle reference chronology potentially reflecting a diet shift, which is known to occur in white sharks (Tricas and McCosker 1984, Estrada et al. 2006, Hussey et al. 2012). Diet shifts over ontogeny have also been observed in the $\Delta^{14}C$ chronologies of tiger (*Galeocerdo cuvier*) sharks, where assay samples from the juvenile portion of the vertebrae aligned with the coral reference chronologies while that of adults were phase lagged and were more closely aligned with the porbeagle chronology (Kneebone et al. 2008).

The percentage of organic and inorganic material can vary along the vertebrae, leading to unequal contributions from different carbon sources (Hamady et al. 2014), and therefore it has been suggested that collagen (the organic portion) should be isolated to obtain an unbiased (by DIC) $\Delta^{14}C$ value (Kerr et al. 2006, Hamady et al. 2014). Kerr et al. (2006) conducted a preliminary study using replicate samples from three individuals to compare the effects of demineralization, and determined that untreated samples were enriched in ^{14}C relative to the demineralized samples. The opposite effect was observed in the current study, whereby the untreated samples were more depleted in ^{14}C than the demineralized samples from the original study (Kerr et al. 2006). The degree of mineralization within shark vertebrae varies by species (Porter et al. 2006), indicating that species-specific studies are required to examine the effect of demineralization on $\Delta^{14}C$ values in elasmobranch vertebrae. Currently, there is no consensus on the requirement for demineralization, and bomb radiocarbon analysis has been successfully tested on multiple species both with (Ardizzone et al. 2006, Kerr et al. 2006, Andrews et al. 2011, Hamady et al. 2014) and without (Campana et al. 2002, Francis et al. 2007, Passerotti et al. 2014) performing demineralization prior to analyzing the vertebrae for

$\Delta^{14}C$. As there are likely differences in age and growth parameters among populations of a species, a systematic study examining the effects of demineralization on the $\Delta^{14}C$ value of vertebral tissue in sharks is required. A standard sample preparation protocol would benefit cross study comparisons.

The $\Delta^{14}C$ values for the SWI white sharks were relatively enriched compared to $\Delta^{14}C$ values for white sharks from the NWA and NEP in both the current and original studies (Kerr et al. 2006, Hamady et al. 2014, Andrews and Kerr 2015), confirming that differences in $\Delta^{14}C$ values among the three populations were not due to methodological differences. Direct comparison between $\Delta^{14}C$ values for the individual sharks from the NWA in this study and the original study (Hamady et al. 2014) could not be made, as samples were not taken from the exact same locations in the vertebrae. The $\Delta^{14}C$ values from the current study were within the range of $\Delta^{14}C$ values reported by Hamady et al. (2014), however, suggesting that there is little to no effect of demineralization on $\Delta^{14}C$ values in white shark vertebrae. The difference in $\Delta^{14}C$ values for NEP white sharks between the current study and the original study may be a result of samples being extracted from a slightly different location on the vertebrae for this study compared to Kerr et al. (2006).

The amplitude of the $\Delta^{14}C$ values in the coral from Watamu Reef is greater and the timing of the rapid increase of ^{14}C slightly lags that of coral reef chronologies in the Atlantic and Pacific Oceans (Druffel and Linick 1978, Druffel 1989, Druffel et al. 2001, Roark et al. 2006). However, two individuals (one each from NEP and NWA) had good alignment with the Watamu Reef coral reference chronology (WH 1, WS 100). The earliest band formed in specimens WS 57 and WS 105 were not sampled, but, the two

assayed samples from each specimen (ages 31 and 66, respectively) were phased lagged and more in line with the porbeagle reference chronology, possibly indicating that these two individuals underwent a diet shift. The highly depleted $\Delta^{14}C$ values for WH 3 and WH 17 were not expected, but highly depleted $\Delta^{14}C$ values were also reported for a white shark (WH 6) in the NEP (Kerr et al. 2006). While we could not align WH 17 with either reference chronology, the timing of its increase in $\Delta^{14}C$ aligns with the rapid increase of $\Delta^{14}C$ observed in the reference chronologies. White sharks have been documented using deep water carbon sources in the NEP which is likely influencing the $\Delta^{14}C$ values in this region and further complicating the interpretation of these specimens (Kerr et al. 2006, Andrews and Kerr 2015). The absolute differences in $\Delta^{14}C$ values among populations are likely due to depth and water mixing rates of the different ocean basins (Weidman and Jones 1993, Kalish 1995, Campana 1999), which is supported by the $\delta^{13}C$ variability observed in the sampled white sharks (Table 3.2). Individual migration patterns and changes in migration/residency throughout ontogeny could also contribute to the recorded variation in $\delta^{13}C$ values. The highly depleted $\delta^{13}C$ value for WH 17 (-29.8‰) was unexpected given the range of $\delta^{13}C$ values measured in this specimen and this value is likely an anomaly (due to measurement error). Further investigation is required to determine if the highly depleted $\Delta^{14}C$ values in the NEP are a common occurrence.

Age estimates for white sharks from the SWI in this study indicate the maximum age (30-38 years) is older than previously estimated (13 years, 373 cm PCL) by Wintner and Cliff (1999). The Wintner and Cliff (1999) study, however, did not include larger sharks such as those used in the current study. These new maximum ages for SWI sharks agree with those observed in the NEP (~30; Andrews and Kerr 2015) but are younger

than that determined for the NWA (~73, 44; Hamady et al. 2014, Natanson and Skomal 2015). The differences in the maximum age among populations are likely due to the specimens available (i.e., Figure 3.5, the number of large animals and the largest sharks included) and not due to absolute differences in the maximum age among populations, although this needs to be confirmed. Additionally, it has been suggested that males are older than females at similar body sizes (Hamady et al. 2014); therefore the lack of large males in this study could have contributed to the lower maximum age.

Dudley and Simpfendorfer (2006) determined that the size at 50% maturity for male white sharks captured in the KZN beach protection program was 344 cm FL; using the growth curve from the current study, the age at 50% maturity for males is 20 years old, whereas using the previous growth curve (Wintner and Cliff 1999) the age at 50% maturity would be 12 years old. The minimum age of mature individuals in this study (16 (312 cm FL) and 30 (487 cm FL) years for males and females, respectively) was greater than previous estimates of 8-10 years (325-352 cm FL; males) and 12-13 years (390 cm FL females; Wintner and Cliff 1999). However, the age of the smallest mature white shark in this study is slightly lower than the age of maturity for white sharks in the NWA (26 (352 cm FL) and 33 (417 cm FL) years for males and females, respectively; Natanson and Skomal 2015). Due to the range in size, 271-365 cm FL (males) and 390-464 cm FL (females), at maturity reported for white sharks globally (Cailliet et al. 1985, Francis 1996, Pratt 1996, Wintner and Cliff 1999, Tanaka et al. 2011) and the relatively small sample size, it is likely that there is an overlap in the age at maturity between the two populations.

Accepting that the largest individuals included in previous studies that used x-ray

analysis were smaller than those included in the current study, growth curve parameters estimated that white sharks in the SWI are slower growing than previously suggested by Cailliet et al. (1985), Wintner and Cliff (1999), and Tanaka et al. (2011; Figure 3.5). The growth curve parameters from the current study were similar to those recently estimated for white sharks in the NWA using validated band pair counts (Hamady et al. 2014, Natanson and Skomal 2015; Figure 3.5), providing additional support for these values. The estimation of L_∞ was limited by the small number of large individuals included in this study, which led to wider confidence intervals. Including additional large individuals would be required to provide greater confidence in the L_∞ estimate.

A recent population estimate (Towner et al. 2013) determined that while the population of white sharks in Gansbaai, South Africa, is larger than at other aggregation sites, there has not been a marked increase in population size since a previous population estimate undertaken 16 years prior (Cliff et al. 1996b). Given the evidence for a low rebound potential for white sharks (Smith et al. 1998), the higher ages at maturity found in this study could provide a plausible explanation for why this population has not recovered. Regional management plans for this species should account for slower growth, later age at maturity and longer life spans.

REFERENCES

Akaike H (1973) Information theory as an extension of the maximum likelihood principle. In: Petrov BN and Csaki F (eds) Proceedings of the second international symposium on information theory. Akademiai Kiad, Budapest, pp 267-281

Andrews AH, Kerr LA (2015) Validated age estimates for large white sharks of the northeastern Pacific Ocean: altered perceptions of vertebral growth shed light on complicated bomb $\Delta^{14}C$ results. Environ Biol Fishes 98: 971-978

Andrews AH, Natanson LJ, Kerr LA, Burgess GH, Cailliet GM (2011) Bomb radiocarbon and tag-recapture dating of sandbar shark (*Carcharhinus plumbeus*). Fish Bull 109: 454-465

Ardizzone D, Cailliet GM, Natanson LJ, Andrews AH, Kerr LA, Brown TA (2006) Application of bomb radiocarbon chronologies to shortfin mako (*Isurus oxyrinchus*) age validation. Environ Biol Fish 77: 355-366

Beamish RJ, McFarlane GA (1983) The forgotten requirement for age validation in fisheries biology. Trans Am Fish Soc 112: 735–743

Baty F, Delignette-Muller ML (2011) nlstools: tools for nonlinear regression diagnostics. R package version 0.0-11. R Foundation for Statistical Computing, Vienna

Broecker WS, Peng TH (1982) Tracers in the sea. Lamont-Doherty Geological Observatory, Palisades, New York, 690 p.

Burnham KP, Anderson DR (2002) Model selection and multi-model inference: a practical information-theoretic approach. 2nd Edn. Springer-Verlag, New York.

Cailliet GM, Andrews AH (2008) Age-validated longevity of fishes: its importance for sustainable fisheries. In: Tsukamoto K, Kawamura T, Takeuchi T, Beard Jr TD, Kaiser MJ (eds) Fisheries for global welfare and environment, 5th World Fisheries Congress. Terrapub, Tokyo, pp 103-120

Cailliet GM, Goldman KJ (2004) Age determination and validation in chondrichthyan fishes. In: Carrier JC, Musick J, Heithaus MR (eds) Biology of Sharks and their Relatives. CRC Press, New York, pp 399-447

Cailliet GM, Natanson LJ, Welden BA, Ebert DA (1985) Preliminary studies on the age and growth of the white shark (*Carcharodon carcharias*), using vertebral bands. Mem S Cal Acad Sci 9: 49-60

Cailliet GM, Smith WD, Mollet HF, Goldman KJ (2006) Age and growth studies of chondrichthyan fishes: the need for consistency in terminology, verification, validation, and growth function fitting. Environ Biol Fish 77: 211-228

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188: 263–297

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol 59: 197-242

Campana SE (2014) Age determination of elasmobranchs, with special reference to Mediterranean species: a technical manual. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 94. Rome, FAO

Campana SE, Natanson LJ, Myklevoll S (2002) Bomb dating and age determination of large pelagic sharks. Can J Fish Aquat Sci 59: 450-455

Casey JG, Pratt Jr. HL, Stillwell CE (1985) Growth of the sandbar shark (*Carcharhinus plumbeus*) from the Western North Atlantic. Can J Fish Aquat Sci 42: 963-975
Cliff G, Dudley SFJ (2011) Reducing the environmental impact of shark control programs: a case study from KwaZulu-Natal, South Africa. Mar Freshw Res 62: 700-709
Cliff G, Dudley SFJ, Jury MR (1996a) Catches of white sharks in KwaZulu-Natal, South Africa and environmental influences. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 351-362
Cliff G, van der Elst RP, Govender A, Witthuhn TK, Bullen EM (1996b) First estimates of mortality and population size of white sharks on the South African coast. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 393–400
Druffel ERM (1989) Decade time scale variability of ventilation in the north Atlantic: high precision measurements of bomb radiocarbon in banded corals. J Geophys Res 94: 3271–3285
Druffel EM, Linick TW (1978) Radiocarbon in annual coral rings of Florida. Geophys Res Lett 5:913-916
Druffel ERM, Griffin S, Guilderson TP (2001) Changes of subtropical north Pacific radiocarbon and correlation with climate variability. Radiocarbon 43: 15–25
Dudley SFJ, Cliff G, Zungu MP, Smale MJ (2005) Sharks caught in protective gill nets off KwaZulu-Natal, South Africa. 10. The dusky shark, *Carcharhinus obscurus* (Lesueur 1818). Afr J Mar Sci 17: 107-127
Dudley SFJ, Simpfendorfer CA (2006) Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. Mar Freshw Res 57: 225-240
Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeding ecology in white sharks. Ecology 87: 829-834
Francis MP (1996) Observations on a pregnant white shark with a review of reproductive biology. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 157-172
Francis MP, Campana SE, Jones CM (2007) Age under-estimation in New Zealand porbeagle sharks (*Lamna nasus*): is there an upper limit to ages that can be determined from shark vertebrae? Mar Freshw Res 58: 10-23
Fry B (1988) Food web structure on Georges Bank from stable C, N and S isotopic composition. Limnol Oceanogr 33: 1182–1190
Goldman KJ, Cailliet GM, Andrews AH, Natanson LJ (2012) Assessing the age and growth of chondrichthyan fishes. In: Carrier J, Musick JA, Heithaus MR (eds) Biology of Sharks and their Relatives. CRC Press, Boca Raton, pp 423- 451
Gordon AL (1986) Interocean exchange of thermocline water. J Geophys Res 91: 5037-5046
Grumet NS, Guilderson TP, Dunbar RB (2002) Pre-bomb radiocarbon variability inferred from a Kenyan coral record. Radiocarbon 44: 591-590
Hamady LL, Natanson LJ, Skomal GB, Thorrold SR (2014) Vertebral bomb radiocarbon suggests extreme longevity in white sharks. PLoS ONE 9: e84006

Hussey NE, McCann H, Cliff G, Dudley SFJ, Wintner SP et al. (2012) Size-based analysis of diet and trophic position of the white shark (*Carcharodon carcharias*) in South African waters. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp 27-50

Huveneers C, Stead J, Bennett MB, Lee KA, Harcourt RG (2013) Age and growth determination of three sympatric wobbegong sharks: How reliable is growth band periodicity in Orectolobidae? Fish Res 147: 413-425

Kalish JM (1993) Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet Sci Lett 114: 549-554

Kalish JM (1995) Radiocarbon and fish biology. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in Fish Otolith Research. University of South Carolina Press, Columbia, SC, pp 637-653

Kerr LA, Andrews AH, Cailliet GM, Brown TA, Coale KH (2006) Investigations of $\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$ in vertebrae of white shark (*Carcharodon carcharias*) from the eastern North Pacific Ocean. Environ Biol Fish 77: 337-353

Kneebone J, Natanson LJ, Andrews AL, Hunt Howell W (2008) Using bomb radiocarbon analyses to validate age and growth estimates for the tiger shark, *Galeocerdo cuvier*, in the western North Atlantic. Mar Biol 154: 423-434

Lai HL, Gunderson DR (1987) Effects of ageing errors on estimates of growth mortality and yield per recruit for walleye, Pollock (*Theragra chalcogramma*). Fish Res 5: 287-302

Natanson LJ, Cailliet GM (1990) Vertebral growth zone deposition in Pacific angle sharks. Copeia 4: 1133-1145

Natanson LJ, Skomal GB (2015) Age and growth of the white shark, *Carcharodon carcharias*, in the western North Atlantic Ocean. Mar Freshw Res 66: 387-398

Natanson LJ, Mello JJ, Campana SE (2002) Validated age and growth of the porbeagle shark, *Lamna nasus*, in the western north Atlantic Ocean. Fish Bull 100: 266-278

Natanson LJ, Wintner SP, Johansson F, Piercy A, Campbell P et al. (2008) Ontogenetic vertebral growth patterns in the basking shark *Cetorhinus maximus*. Mar Ecol Prog Ser 361: 267-278

Passerotti MS, Carlson JK, Piercy AN, Campana SE (2010) Age validation of great hammerhead shark (*Sphyrna mokarran*), determined by bomb radiocarbon analysis. Fish Bull 108: 346–351

Passerotti MS, Andrews AH, Carlson JK, Wintner SP, Goldman KJ et al. (2014) Maximum age and missing time in the vertebrae of sand tiger shark (*Carcharias taurus*): validated lifespan from bomb radiocarbon dating in the western North Atlantic and southwestern Indian Oceans. Mar Freshw Res 65: 674-687

Porter ME, Beltrán JL, Koob TJ, Summers AP (2006) Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondricthyes). J Exp Biol 209: 2920-2928

Pratt HL (1996) Reproduction in the male white shark. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 131-138

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ridewood WG (1921) On the calcification of the vertebral centra in sharks and rays. Phil Trans R Soc B 210: 311-407
Roark EB, Guilderson TP, Dunbar RB, Ingram BL (2006) Radiocarbon based ages and growth rates: Hawaiian deep sea corals. Mar Ecol Prog Ser 327: 1-14
Schnute J (1981) A versatile growth model with statistically stable parameters. Can J Fish Aquat Sci 38: 1128-1140
Smale M, Cliff G (2012) White sharks and cephalopod prey: Indicators of habitat use? In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp 51-57
Smith SE, Au DW, Show C (1998) Intrinsic rebound potentials of 26 species of Pacific sharks. Mar Freshw Res 49: 663-678
Stuiver M, Polach HA (1977) Discussion reporting of ^{14}C data. Radiocarbon 19: 355-363
Tanaka S, Kitamura T, Mochizuki T, Kofuji K (2011) Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kahima-nada, Japan. Mar Freshw Res 62: 548-556
Towner AV, Wcisel MA, Reisinger RR, Edwards D, Jewell OJD (2013) Gauging the threat: the first population estimate for white sharks in South Africa using photo identification and automated software. PLoS ONE 8: e66035
Tricas TC, McCosker JE (1984) Predatory behavior of the white shark (*Carcharodon carcharias*), with notes on its biology. Pro Calif Acad Sci 43: 221-238
Uchida S, Toda M, Teshima K, Yano K (1996) Pregnant white sharks and full-term embryos from Japan. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 139-155
Weidman CB, Jones GA (1993) A shell-derived time history of bomb ^{14}C on Georges Bank and its Labrador Sea implications. J Geophys Res 98: 14577-14588
Wintner SP, Cliff G (1999) Age and growth determination of the white shark, *Carcharodon carcharias*, from the east coast of South Africa. Fish Bull 97: 153-169

Table 3.1 Summary of white shark (*Carcharodon carcharias*) vertebral samples for $\Delta^{14}C$ analysis. SWI, southwest Indian Ocean (n=4); NEP, northeastern Pacific Ocean (n=3); NWA, northwestern Atlantic Ocean (n=3). Ages were estimated from band pair counting. Birth year was determined as year collected minus estimated age. Bands sampled are the number of bands extracted and analyzed for $\Delta^{14}C$.

Shark ID	Region	Sex	Fork length (cm)	Year collected	Estimated age (yr)	Birth year	Bands sampled
Gansbaai	SWI	F	537	1987	31	1956	3
Kenya96	SWI	F	487	1996	30	1966	1
IFA91016	SWI	M	329	1991	18	1973	3
TRA92004	SWI	M	414	1992	38	1954	1
WS 1	NEP	M	365	1978	21	1957	1
WS 3	NEP	M	214	1968	6	1962	1
WS 17	NEP	M	429	1982	46	1936	3
WS 57	NWA	M	441	1981	31	1950	2
WS 100	NWA	M	223.5	1968	8	1960	1
WS 105	NWA	M	493	1986	66	1920	2

Table 3.2 Results of bomb radiocarbon dating for white sharks (*Carcharodon carcharias*). Estimated age was based on band pair counting. Band sampled is based-on band pair counting. Year of formation was calculated as the year of birth plus the estimated age of the band sampled.

Shark ID	Fork length (cm)	Estimated age (yr)	Band sampled	Year of formation	$\delta^{13}C$	$\Delta^{14}C$	CV of assigned band pair formation[1]
Southwest Indian Ocean							
Gansbaai	537	31	1.5	1957.5	-16.6	-8	5.1
			3	1959	-11.3	12.3	4.9
			9.5	1965.5	-11.2	23.7	3.7
IFA91016	329	18	1	1974	-12.2	26.3	3.0
			6	1979	-11.4	78.7	2.1
			15	1988	-12.7	74.7	0.6
Kenya96	487	30	2	1968	-10.1	41.4	4.9
TRA92004	414	38	1.5	1955.5	-9	-0.6	6.4
Northeast Pacific Ocean							
WS 1	365	21	0-1	1957.5	-12.3	-43.29	
WS 3	214	6	1	1963	-17.9	-181.16	
WS 17	429	46	1-2	1937.5	-13.5	-148.94	
			21-25	1959	-29.8	-181.16	
			36-40	1974	-13.1	-3.67	
Northwest Atlantic Ocean							
WS 57	441	31	7-9	1958	-13.4	-68.2	
			17-19	1968	-14	4	
WS 100	223.5	8	0-1	1960.5	-13.6	-22.7	
WS 105	493	66	37-40	1958.5	-12.7	-77.1	
			48-55	1971.5	-13.2	10.9	

[1] CV =17.4%

Table 3.3 Summary of bomb radiocarbon dating results for white sharks (*Carcharodon carcharias*) using untreated vertebral samples from this study compared to demineralized samples from previous studies (northeast Pacific Ocean (NEP) (Kerr et al. 2006); northwest Atlantic Ocean (NWA) (Hamady et al. 2014)). Estimated age is that based on band pair counting from the current study. Estimated bomb $\Delta^{14}C$ age determined in the NEP and the NWA by bomb radiocarbon analysis presented in the original studies (Kerr et al. 2006, Hamady et al. 2014); adjusted age for the NEP sharks accounts for missing annuli according to Andrews and Kerr (2014).

Shark ID	Current study			Original Study		
	Estimated age (yr)	Band sampled	$\Delta^{14}C$ (untreated)	Estimated bomb $\Delta^{14}C$ age (adjusted age)	Band sampled	$\Delta^{14}C$ (demineralized)
Northeast Pacific Ocean						
WS 1	21	0.5	-43.29	7 (>22)	1	-59.7
WS 3	6	1	-181.16	3 (12)	1	-72.2
WS 17	46	1.5	-148.94	18 (30, 37+)	2	-74.1
		23	-181.16		7	-65.6
		38	-3.67		12	-29.2
					17	34.7
Northwest Atlantic Ocean						
WS 57	31	8	-68.2	44	1	-54.97
		18	4		17	-62.25
WS 100	8	0.5	-22.7	9	1.5	1.83
WS 105	66	38.5	-77.1	73	1	-60.34
		52	10.9		42	-57.07

Table 3.4 Relative goodness of fit for each candidate growth model for white sharks (*Carcharodon carcharias*) from the southwestern Indian Ocean. Models are ranked from best to worst fit. Abbreviations: k= total number of regression parameters, including the error term; AIC_c= second-order Akaike information criterion; Δ_i= Akaike difference; w_i= Akaike weight; and LL= log-likelihood

Model	k	AIC_c	Δ_i	w_i	LL
Gompertz	4	595.20	0	0.31	-293.20
Logistic	4	595.23	0.03	0.31	-293.22
von Bertalanffy	4	595.31	0.11	0.29	-293.26
Schnute General Model	5	597.62	2.42	0.09	-293.20

Table 3.5 Growth model parameters for white sharks (*Carcharodon carcharias*) from the southwest Indian Ocean based on vertebral age estimates. The bootstrap 95% confidence intervals for each parameter are indicated in parentheses below the number. Abbreviations: a= a constant (time^{-1}) describing the constant relative rate of the relative growth rate; b= a dimensionless constant describing the incremental relative rate of the relative growth rate; L_1= estimated length at age 1: L_2= estimated length at age 38; L_∞= asymptotic fork length; L_0=length at birth; t_0= theoretical age at zero length. All lengths presented are given in fork length (cm).

Model	***a***	***b***	L_1	L_2	L_∞	L_0	t_0
Gompertz	0.061	0	144.90	436.80	496.77	134.08	-
	(0.02-0.10)		(113.18-176.03)	(388.80-502.89)	(404.37-947.57)	(98.85-168.03)	
Logistic	0.091	-1	150.41	434.77	466.03	141.28	-
	(0.050-0.14)		(121.32-179.90)	(384.83-501.17)	(392.62-692.53)	(110.00-173.10)	
von Bertalanffy	0.03	1	138.47	439.96	583.53	124.65	-7.86
	(0.00-0.07)		(99.28-176.67)	(390.92-501.43)	(-440.06-2018)	(77.88-168.54)	(-23.02 to -2.98)
Schnute General Model	0.07	-0.18	145.97	436.36	489.15	135.53	-
	(-0.12-0.32)	(-7.06-6.30)	(86.59-175.14)	(384.50-498.95)	(385.39-1006.79)	(63.18-168.60)	

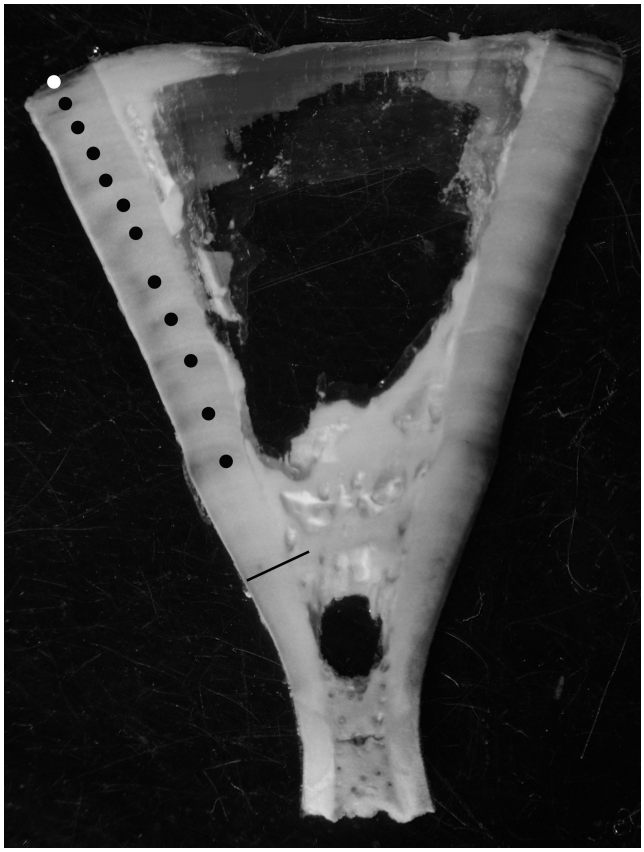

Figure 3.1 Vertebral section from a 292 cm FL male white shark (*Carcharodon carcharias*) aged 12 years from band pair counts. Circles indicate translucent bands. Black line indicates birth mark.

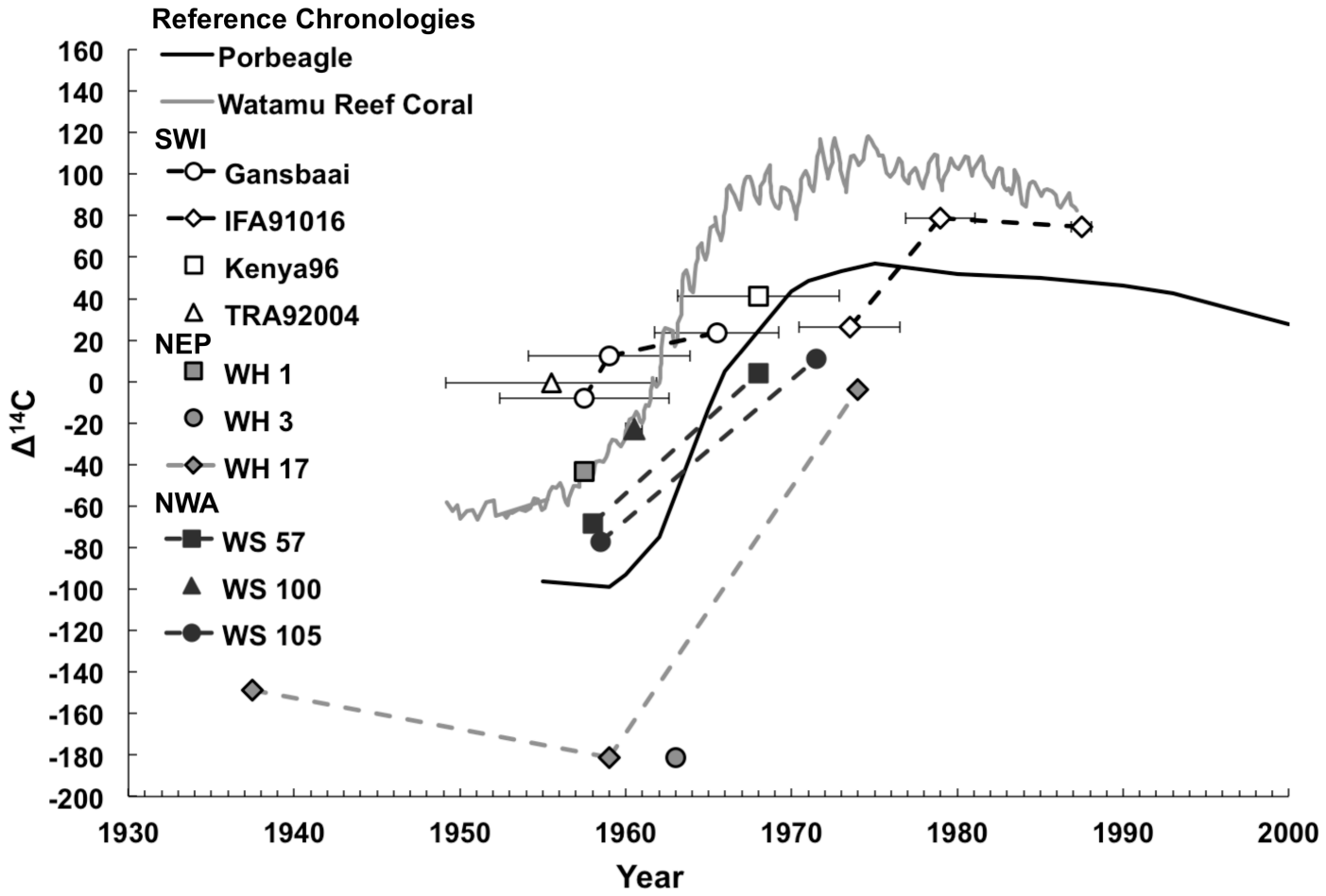

Figure 3.2 $\Delta^{14}C$ values of white sharks from the southwest Indian Ocean (SWI), northeast Pacific Ocean (NEP) and northwest Atlantic Ocean (NWA) compared to two $\Delta^{14}C$ reference chronologies: the porbeagle shark from the NWA (Campana et al. 2002) and a hermatypic coral from Watamu Reef off the coast of Kenya (Grumet et al. 2002). Horizontal error bars represent uncertainty associated with age estimation from growth bands (CV=17.4%).

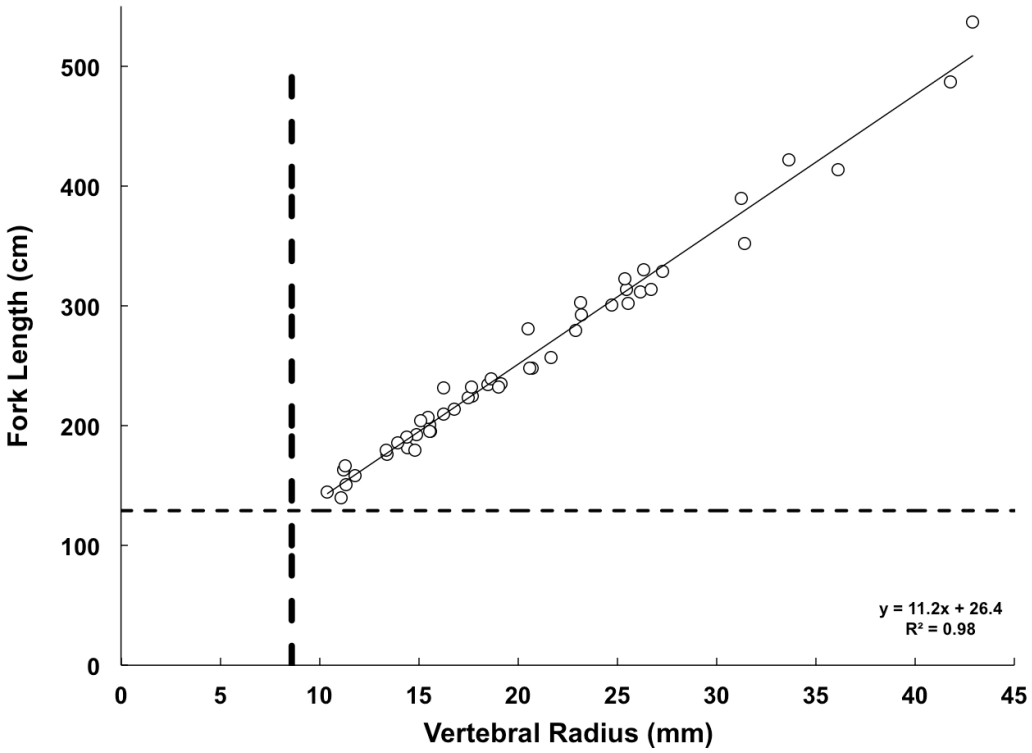

Figure 3.3 Relationship between vertebral radius and fork length for white sharks (*Carcharodon carcharias*) in the southwest Indian Ocean. The horizontal line represents the size at birth calculated in this study (129 cm) and the vertical line represents the mean radius of the birth mark (8.6 mm, n=36).

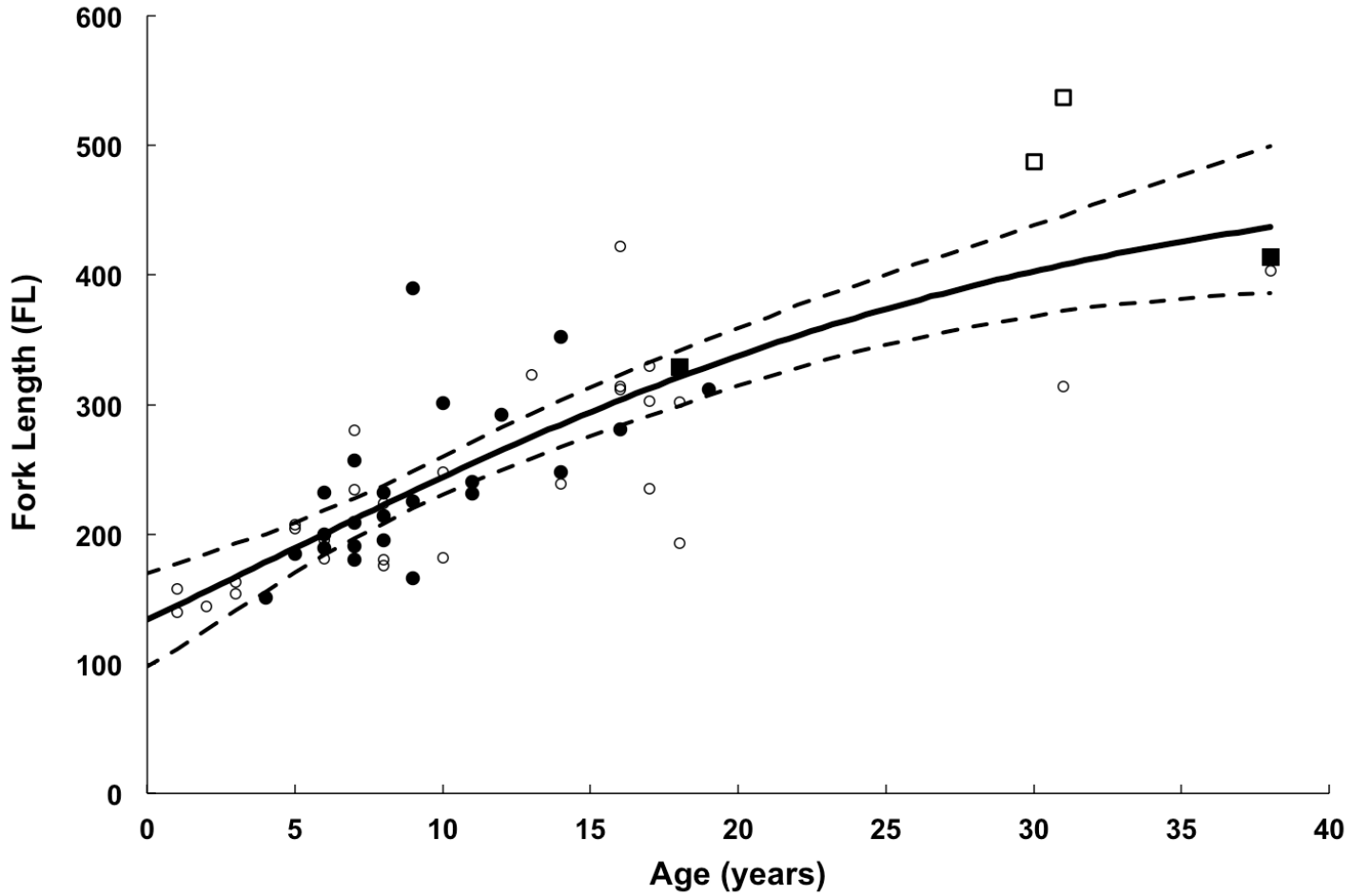

Figure 3.4 Gompertz growth curve based on vertebral band pair counts for white sharks (*Carcharodon carcharias*) from the southwest Indian Ocean (SWI). Open circles (females, n=30) and black circles (males, n=25) indicate length at age data for each individual. The solid black line is the Gompertz growth model with 95% confidence intervals (dashed lines).

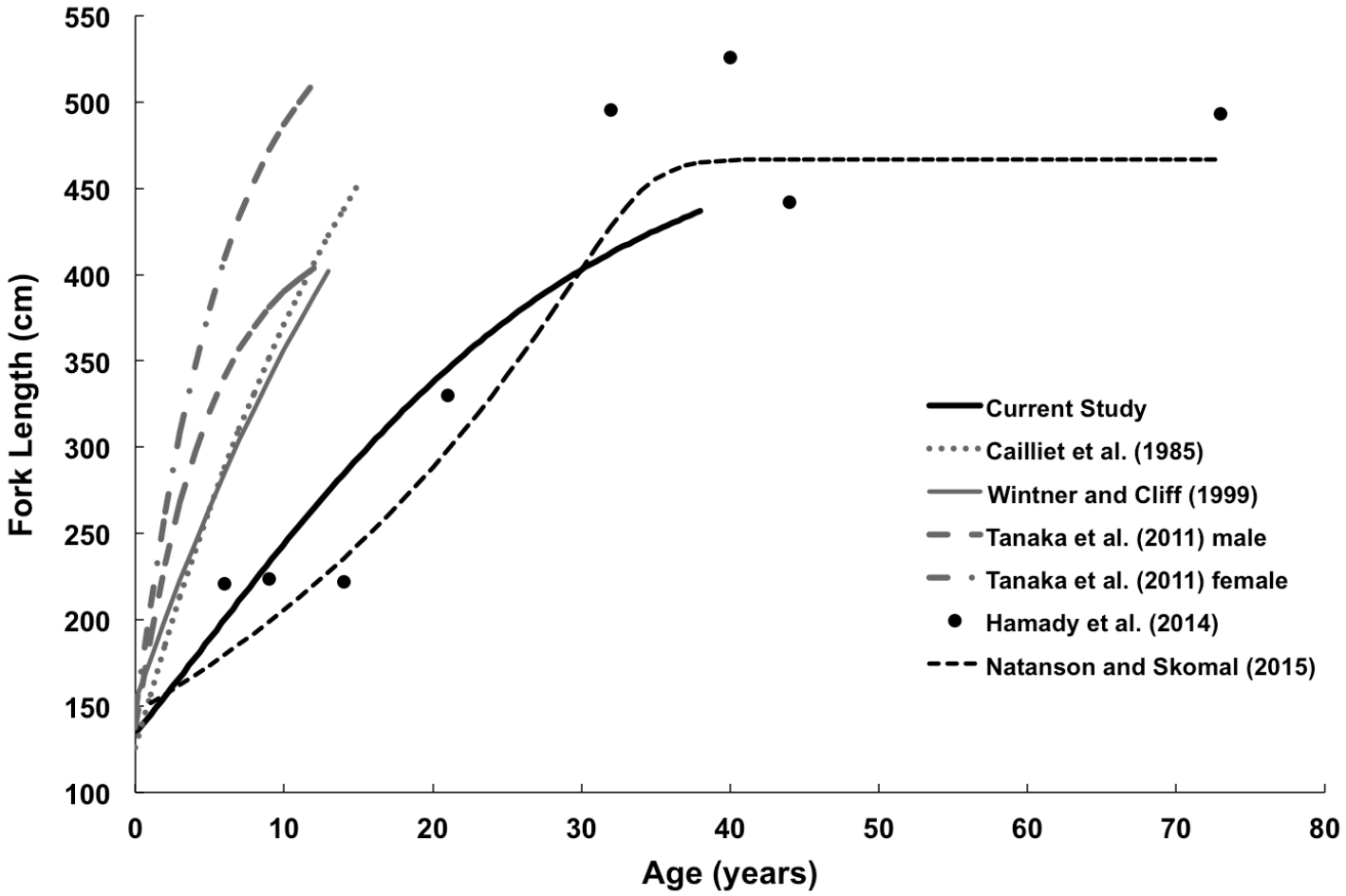

Figure 3.5 Comparative white shark (*Carcharodon carcharias*) growth curves. Black solid line is Gompertz growth curve from this study. Dashed black line is the Schnute general model growth curve from Natanson and Skomal (2015). Solid light gray line is von Bertalanffy growth curve from Cailliet et al. (1985). Light gray dashed line is von Bertalanffy growth curve from Wintner and Cliff (1999). Dark gray (solid-female, dashed-male) is von Bertalanffy growth curve from Tanaka et al. (2011).

Table S3.1 Results of bomb radiocarbon dating for white sharks (*Carcharodon carcharias*). Year of formation (YOF) was calculated as the year of birth plus the estimated age of the band sampled. Estimated age was based on band pair counting. YOF (coral) is the year of formation when shifted to align with the Watamu Reef coral reference chronology and the resulting age. YOF (porbeagle) is the year of formation when shifted to align with the porbeagle (*Lamna nasus*) reference chronology and the resulting age.

Shark ID	YOF	Estimated age (yr)	YOF (coral)	Estimated age (yr- coral)	YOF (porbeagle)	Estimated age (yr-porbeagle)
Kenya96	1968	30	1964	34	1970	28
Gansbaai	1957.5	31	1961	27.5	1965	23.5
	1959		1962.5		1966.5	
	1965.5		1969		1973	
IFA91016	1973.5	18	1963	29	1970	22
	1979		1968		1975	
	1987.5		1977		1984	
TRA92004	1955.5	38	1961	32.5	1966	27.5

Table S3.2 Relative goodness of fit for each candidate growth model for white sharks (*Carcharodon carcharias*) from the southwestern Indian Ocean. Models are ranked from best to worst fit. Abbreviations: k= total number of regression parameters, including the error term; AIC_c= second-order Akaike information criterion; Δ_i= Akaike difference; w_i= Akaike weight; and LL= log-likelihood **A-** Using ages adjusted to porbeagle (*Lamna nasus*) reference chronology for the four bomb radiocarbon specimens. **B-** Using ages adjusted to Watamu Reef coral reference chronology for the four bomb radiocarbon specimens.

A

Model	k	AIC_c	Δ_i	w_i	LL
Logistic	4	600.78	0	0.38	-295.99
Gompertz	4	601.02	0.24	0.33	-296.11
von Bertalanffy	4	601.45	0.67	0.27	-296.33

B

Model	k	AIC_c	Δ_i	w_i	LL
von Bertalanffy	4	598.28	0	0.30	-294.74
Gompertz	4	598.29	0.01	0.30	-294.75
Logistic	4	598.43	0.15	0.28	-294.82
Schnute General Model	5	600.69	2.41	0.09	-294.73

Table S3.3 Growth model parameters for white sharks (*Carcharodon carcharias*) from the southwest Indian Ocean based on vertebral age estimates. **A**- Ages for specimens sampled for bomb radiocarbon assay adjusted to porbeagle (*Lamna nasus*) reference chronology. **B**- Ages for specimens sampled for bomb radiocarbon assay adjusted to Watamu Reef coral reference chronology. The bootstrap 95% confidence intervals for each parameter are indicated in parentheses below the number. Abbreviations: a= a constant (time^{-1}) describing the constant relative rate of the relative growth rate; b= a dimensionless constant describing the incremental relative rate of the relative growth rate; L_1= estimated length at age 1: L_2= estimated length at age 38; L_∞= asymptotic fork length; L_0=length at birth; t_0= theoretical age at zero length. All lengths presented are given in fork length (cm). Error indicates model would not converge.

	Model	a	b	L_1	L_2	L_∞	L_0	t_0
A	Gompertz	0.07 (0.02-0.12)	0	139.00 (105.87-176.04)	434.29 (374.98-530.49)	472.54 (379.98-1093.68)	127.87 (89.52-169.21)	-
	Logistic*	0.11 (0.05-0.17)	-1	143.67 (110.92-178.29)	428.36 (368.82-516.58)	446.03 (371.04-708.05)	135.45 (98.42-171.10)	-
	von Bertalanffy	0.04 (-0.01 to 0.08)	1	131.00 (91.10-171.96)	439.70 (378.94-527.27)	545.22 (-448.54 to 1664.39)	118.13 (65.79-164.20)	-6.73 (-23.62 to -2.22)
	Schnute General Model				Error			
B	Gompertz	0.07 (0.02-0.11)	0	143.06 (110.46-177.81)	428.58 (380.89-497.62)	477.01 (391.14-1312.21)	133.63 (95.33-170..50)	-
	Logistic	0.10 (0.05-0.15)	-1	149.14 (118.98-179.54)	423.94 (376.71-487.68)	448.11 (381.28-660.79)	140.57 (107.66-172.56)	-
	von Bertalanffy*	0.03 (-0.01-0.08)	1	136.49 (95.86-180.57)	433.59 (381.86-497.05)	555.14 (-472.59 to 2032.29)	124.67 (70.44-174.71)	-7.72 (-21.52 to -2.98)
	Schnute General Model	0.05 (-0.15-0.32)	0.80 (-7.15-6.71)	131.38 (75.00-176.33)	431.31 (374.91-503.86)	510.54 (374.70-1027.84)	128.70 (48.00-171.01)	-

*Best fit model

CHAPTER 4: INCORPORATING STABLE ISOTOPES INTO A MULTIDISCIPLINARY FRAMEWORK TO IMPROVE DATA INFERENCE AND THEIR CONSERVATION AND MANAGEMENT APPLICATION[3]

INTRODUCTION

Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide a powerful chemical tracer to examine diverse questions in ecology; from ecological characteristics of individual organisms, including diet and resource use (DeNiro and Epstein 1978, DeNiro and Epstein 1981, Minagawa and Wada 1984, Peterson and Fry 1987), niche width and niche overlap (Heithaus et al. 2013, Kiszka et al. 2015), individual specialism (Hückstädt et al. 2012, Rosenblatt et al. 2015), movement and migration (Best and Schell 1996, Hobson 1999, Phillips et al. 2009), to the length of, structure and interactions of organisms within food webs (Hobson and Welch 1992, Hussey et al. 2014), to name but a few. These tools are now being applied in the study of all types of species from bacteria (Boschker and Middelburg 2002) to the largest terrestrial and aquatic predators (Hilderbrand et al. 1996, Herman et al. 2005), at individual, community, and ecosystem levels, and have particular value for studies on species that are obscure (Navarro et al. 2014), of high commercial value (Estrada et al. 2005, Johnson and Schindler 2009), and/or critically endangered (Navarro et al. 2009, Seminoff et al. 2012). Importantly, through exponential growth in the application of stable isotopes over recent years and practical and theoretical advancements (Gannes et al. 1997, Wolf et al. 2009), there has been broad recognition of their relevance for informing management (Rubenstein and Hobson 2004, Ramos et al. 2011, Hussey et al. 2014).

[3] Christiansen HM, Fisk AT, Hussey NE (2015) Incorporating stable isotopes into a multidisciplinary framework to improve data inference and their conservation and management application. Afr J Mar Sci 37: 189-197

The acceptance of stable isotopes as a valuable tool for use by resource managers is a positive step, but this sets a new bar on the quality of data inference and our confidence as a community in their interpretation. To justify the role of stable isotopes as a tool to inform the future management of marine and terrestrial ecosystems, the stable isotope community, as with all disciplines, must systematically and objectively review their methods and approaches to ensure accurate data interpretation. This is particularly pertinent given the number of assumptions inherent with stable isotopes (for example, diet and tissue-specific discrimination factors, variable tissue turnover rates, and physiological effects that vary among individuals and species) (Gannes et al. 1997, Martínez Del Rio et al. 2009, Wolf et al. 2009) and quantitative modeling approaches used (the requirement to select relevant prey items and sources and knowledge of isotopic baselines) (Phillips and Gregg 2003, Moore and Semmens 2008, Parnell et al. 2010, Phillips et al. 2014). Recently, there has been increased use of stable isotopes in conjunction with other established ecological tools, for example compound specific isotope analysis of amino acids (CSIA) (Seminoff et al. 2012, Vander Zanden et al. 2013, Hussey et al. 2015), fatty acids (Kharlamenko et al. 2001, Couturier et al. 2013), trace elements (Werry et al. 2011, Honda et al. 2012), genetics (Clegg et al. 2003, Valenzuela et al. 2009), telemetry (Cunjak et al. 2005, Ceriani et al. 2012, Carlisle et al. 2014, Matich and Heithaus 2014), and measures of organism condition (Hobson et al. 1993, Gannes et al. 1998), to name but a few. This is leading to a multidisciplinary approach that inherently will yield more precise conclusions compared to the use of stable isotopes as a stand-alone tool.

Here, we promote the integration of isotopes within a multidisciplinary context based on a case study of a large, threatened fish, the white shark (*Carcharodon carcharias*). We define multidisciplinary as research in which each discipline (or subdiscipline) makes an individual contribution to the overall research findings. Through ontogenetic stable isotope profiles derived from the juvenile portion of white shark vertebrae, we demonstrate that there are several equally viable explanations for the observed isotopic trends. Each interpretation, however, would differentially impact the species risk designation and management planning actions. The use of techniques from multiple subdisciplines within biology (e.g., genetics, telemetry, trace elements) coincident with these stable isotope data would increase our interpretive ability and hence more effectively inform management for this life-stage. We recommend, where possible, that incorporating stable isotope data in to a multidisciplinary framework will markedly improve their application for conservation and management planning.

CASE STUDY: VERTEBRAL STABLE ISOTOPE PROFILES ($\delta^{15}N$ AND $\delta^{13}C$) OF WHITE SHARKS OFF SOUTHERN AFRICA.

Stable isotope profiles ($\delta^{15}N$ and $\delta^{13}C$) were derived from fifty-eight white shark vertebrae sampled from animals incidentally caught in beach protection nets off KwaZulu-Natal, southeast Africa. In brief, dried vertebrae were sectioned and sample material was drilled from the vertebrae every 1-2 mm along the center of the *corpus calcareum*. Individual age bands were not analyzed and data is presented as sequential sampling throughout ontogeny. This was based on the assumption that similar sized individuals will have similar band thicknesses and therefore the integration time per sequential sampling point will not differ significantly among individuals. The juvenile

portion was defined as samples starting after the angle change (birth) to approximately 200 cm precaudal length (PCL). A linear regression between PCL vs. vertebral radius found that 200 cm PCL corresponded to approximately 17 mm across the vertebral section from the focus. It has been shown that white shark vertebral samples do not require decalcification prior to isotope analysis; therefore no pretreatment was undertaken (Christiansen et al. 2014a).

The juvenile vertebral isotope profiles (both $\delta^{15}N$ and $\delta^{13}C$) of these 58 white sharks, showed markedly distinct trends across time and individuals (Figure 4.1). Values of $\delta^{13}C$ and $\delta^{15}N$ were highly variable among individuals immediately after birth, ranging from -10.9 to -16.5‰ and 12.1 to 17.1‰, respectively (Figure 4.1B, E). Each individual, however, showed consistent isotope values across consecutive sampling points until all individual isotope profiles converged at ~200 cm PCL (Figure 4.1). The consistency and convergence of $\delta^{13}C$ values was more striking than that of $\delta^{15}N$. These trends, both inter-individual differences in $\delta^{13}C$ and $\delta^{15}N$ values between juveniles and consistency in isotope values through time for each individual are notable and identify divergent life history strategies during these early years. This would indicate these are likely management-critical years, as variable life history strategies within the population would require more complex management. The convergence point in isotope values at ~200 cm PCL would suggest these animals then occur in the same food web, based on $\delta^{13}C$, and feed at a similar trophic level, based on $\delta^{15}N$. There are several plausible interpretations that may act independently or in combination, to explain the observed inter-individual variation.

VIABLE EXPLANATIONS FOR OBSERVED WHITE SHARK STABLE ISOTOPE PROFILES

1. Maternal influence

For most live bearing organisms, newborn animal tissues are formed of their mothers' provisions during gestation; hence their stable isotope values reflect those of their mother (i.e. maternal isotopic interference). Knowledge of this maternal-newborn isotopic relationship can consequently be used to retrospectively examine variation in foraging locations among pregnant females. For example, $\delta^{13}C$ and $\delta^{15}N$ values measured in the hair of Northern elephant seal (*Mirounga angustirostris*) pups provided evidence that mothers from two breeding colonies also have distinct foraging habitats (Aurioles et al. 2006). Conversely, this relationship can confound data interpretation of the stable isotope values of newborn/juvenile animals depending on the level of isotopic discrimination between embryo and mother, the rate of elimination of the mother's isotope value, and the incorporation rate of the juvenile's own diet (Matich et al. 2010, Olin et al. 2011). For placental viviparous shark species (those connected by umbilical cord to mother during in utero development), the stable isotope values of near-term and newborn young are typically enriched in both ^{13}C and ^{15}N compared to the mother (McMeans et al. 2009, Vaudo et al. 2010). Over time, these initial high $\delta^{13}C$ and $\delta^{15}N$ values decline as they equilibrate with those of their own diet (Olin et al. 2011), a result of ontogenetic shifts in the diet and habitat use of many marine species driving mature females to consume a different diet and reside in a different habitat to juveniles (Lowe et al. 1996, Alonso et al. 2002, Hussey et al. 2011).

For juvenile white sharks, both $\delta^{13}C$ and $\delta^{15}N$ values generally increased with time following birth. If at birth, the initial values of ^{13}C and ^{15}N were enriched relative to the mother, it would be expected that ^{13}C and ^{15}N would deplete as the isotope values became more representative of the individual's own diet/foraging location (McMeans et al. 2009, Vaudo et al. 2010, Olin et al. 2011). Given that the isotope values of large female white sharks are depleted in ^{13}C, likely related to offshore foraging during gestation (Carlisle et al. 2012, Domeier and Nasby-Lucas 2013, see Figure 4.1A >315cm PCL sharks), it is possible that the observed pattern of isotopic convergence in these juveniles reflects the loss of the maternal isotope value over time. Since vertebrae are metabolically stable (Campana et al. 2002), any maternal influence would be due to growth from maternally derived nutrients in the whole body of the newborn shark (i.e. muscle tissue). Since there are known gradients in $\delta^{13}C$ values between coastal and pelagic waters (France 1995), $\delta^{13}C$ values of juveniles would be expected to increase as they begin feeding in coastal food webs. The range of post-birth $\delta^{13}C$ values observed in juvenile white sharks may represent differences in maternal habitat (i.e. offshore vs. inshore feeding during gestation), rather than actual habitat differences among juveniles.

2. Individual specialists within a generalist population

It has long been recognized that ecological differences in diet and foraging habitat occurs among sex and age classes of species, however, more recently the focus has expanded to include intraspecific niche variation and its potential impact on populations (Bolnick et al. 2002, 2003). Dietary specialization occurs when individuals within a population use a subset of the total available prey base available to that population. Recognition of this behavior has generated new insights into the feeding dynamics and

interactions across a wide variety of taxonomic groups including gastropods, fishes, birds, reptiles, and mammals (Bolnick et al. 2003). Novel stable isotope approaches have now been developed to quantify inter-individual variation in populations that otherwise would be difficult and labor intensive to study (Newsome et al. 2009, Vander Zanden et al. 2010, Matich et al. 2011). Additionally, analysis of incrementally growing structures (e.g. sea otter vibrissae, turtle scute, and shark vertebrae) allows for the study of intra-individual changes in isotope values over time (e.g. from months to entire lifetime).

Individual juvenile white sharks showed relatively consistent stable isotope profiles prior to convergence, resulting in the variation in isotope values between individuals being much greater than within individual variation (Figure 4.1C, F). Under the assumptions of intra-niche variation, this would suggest the potential for individual dietary specialization among juvenile white sharks. This is further supported by the broad range of teleost and elasmobranch prey identified in the diet of juvenile sharks (Hussey et al. 2012). Previous stable isotope profiles of white shark vertebrae from the Northeast Pacific also identified a high degree of dietary specialization, with isotopic trends similar to those observed in our data, but this study was focused on larger individuals (Kim et al. 2012).

3. Multiple nurseries

Carbon stable isotopes have been shown to be effective at discriminating the foraging habitats and migration patterns of animals (France 1995, Hobson 1999). This is based on the premise that carbon stable isotopes in a consumer fractionate conservatively through food webs and thus represent the isotope value of the baseline carbon sources where the animal feeds. In marine systems, pelagic, and offshore food webs are readily

distinguishable because carbon in pelagic systems is driven by photosynthesis and tends to be depleted in ^{13}C. Large-scale latitudinal gradients in stable isotope values can also occur and have been used to identify the habitat and migration of a range of species (Best and Schell 1996, Cherel and Hobson 2007). Off Southern Africa, there is a known gradient of $\delta^{13}C$ values, such that ^{13}C enriches along the Eastern coast from KwaZulu-Natal to the Western Cape (Hill et al. 2006). Moreover, gradients in $\delta^{13}C$ values are documented in the tropical Western Indian Ocean (Ménard et al. 2007) and across the sub-Antarctic front (Best and Schell 1996, Cherel and Hobson 2007).

The large variation in $\delta^{13}C$ values of juvenile white sharks post-birth and their consistency over time prior to convergence could suggest these animals inhabit distinct geographic locations or nurseries. Multiple nurseries could occur either in coastal and/or pelagic waters or could be shared across continents. Transoceanic dispersal events have been recorded for white sharks (Blower et al. 2012), indicating that South African females may give birth in Australian waters or that juveniles are capable of transoceanic migrations (Bruce and Bradford 2008). A sub-adult female white shark was documented making a return migration to Australia from South Africa (Bonfil et al. 2005), however this migration event has yet to be observed in smaller individuals. Alternatively, nurseries could be distributed along the Western Indian Ocean coast (i.e. by latitude). The only large near-term pregnant female white shark observed in the region was caught off Kenya, supporting this hypothesis (Cliff et al. 2000). Furthermore, in Australia, satellite telemetry data has revealed that juvenile white sharks inhabit two distinct nursery areas with few individuals migrating between habitats (Bruce and Bradford 2012).

4. Physiological constraints

The most obvious physiological effect on organismal stable isotope values relates to the effect of fasting. Once an animal fasts, i.e. it no longer consumes food, catabolism occurs leading to preferential excretion of ^{14}N (Hobson et al. 1993). This excretion results in higher $\delta^{15}N$ values in a consumer, which then confounds quantification of the actual diet of that individual. This relationship is likely ubiquitous across species that fast and/or experience periods of low food availability (i.e. poor condition), because most adapt to reduced food intake through identical metabolic processes. The magnitude of the effect of fasting however is thought to be variable. For organisms that use high levels of protein and maintain low lipid stores or have high energetic requirements (e.g. for long distance migrations), the effect is thought to result in a greater change in $\delta^{15}N$ values (Cherel et al. 2005).

The consequences of fasting for a population of a species with highly variable life history dynamics, such as partial migrations, where some individuals remain resident while others commonly migrate, could lead to large among individual variation in $\delta^{15}N$ values even if individuals feed on a similar diet (with similar $\delta^{15}N$ values). There is evidence to support white sharks feeding during offshore migrations (Carlisle et al. 2012); but individuals returning to coastal waters were observed to be in poorer condition, and mixing models indicated a reduced level of foraging in pelagic versus coastal environments (Chapple et al. 2011, Carlisle et al. 2012). The extent of fasting or reduced foraging of white sharks during offshore migrations off Southern Africa is unknown, but variable residency and migration dynamics of animals within the population may explain the observed isotopic patterns. Alternatively, highly variable growth may occur during the juvenile life stage driving physiological variation in the

uptake of stable isotopes, for example through variable fractionation. For Atlantic salmon (*Salmo salar*), varying growth rates contributed to variation in $\delta^{15}N$ values among individuals held under controlled conditions (Trueman et al. 2005). Although $\delta^{13}C$ values are reportedly not affected by periods of fasting (Hobson and Clark 1993), there have been mixed results (Cherel et al. 2005, Williams et al. 2007) and little is known regarding these effects in sharks.

THE BENEFITS OF STABLE ISOTOPES ($\delta^{15}N$ AND $\delta^{13}C$) INCORPORATED INTO A MULTIDISCIPLINARY APPROACH

While the four above-mentioned explanations are all plausible, the implications of each for the management of the white shark population off Southern Africa are highly variable. These management implications are summarized in Figure 4.2, ranked from low to high priority. In the broader stable isotope literature, it is likely that many similar studies exist, where several equally plausible explanations were possible, but only one was argued. In turn, the selected interpretation may have influenced the conservation status of a species or prospective management actions (Bond and Diamond 2011). Indeed, it is only through multidisciplinary approaches, that the initial interpretation of bulk stable isotope data (bulk refers to analysis of whole tissues, as opposed to individual compounds), when used as a stand-alone tool, is being challenged. This certainly does not devalue the application of bulk stable isotopes in ecology; science must advance and build. Importantly though, it reinforces the need for investigators to be cognitive of how they interpret and 'sell their' data and to consider integrating stable isotopes in multidisciplinary frameworks where possible. This is particularly important given the role of science, including stable isotope ecology, in assisting and developing conservation

and management actions. Here, we provide a few examples of recently adopted multidisciplinary isotopic frameworks.

Stable isotope analysis provides a single $\delta^{15}N$ and $\delta^{13}C$ value for a consumer, while CSIA can provide both a $\delta^{15}N$ and $\delta^{13}C$ value of the organism (trophic amino acids) and the baseline of the system (source amino acids) (McClelland and Montoya 2002). This overcomes the limitation of establishing isotopic baselines for the ecosystems an animal inhabits, and is particularly useful for animals that are highly migratory. Through combining these two approaches, it was possible to determine reliable estimates of trophic position (TP; the level an animal feeds in a food web) for two mesopelagic fish families (Myctophidae and Stomiidae) across five global regions (Choy et al. 2012). While bulk $\delta^{15}N$ values produced variable estimates of TP, CSIA revealed that TP was uniform and the observed variation was a result of region specific water body biogeochemistry (Choy et al. 2012). Similarly, stable isotope values of herbivorous green turtles (*Chelonia mydas*) sampled at a nesting ground, suggested that individuals fed over several trophic levels, indicating carnivory (Vander Zanden et al. 2013). CSIA revealed that these results were due to variation in baseline seagrass isotope values between foraging grounds, consequently all individuals fed at a similar TP (Vander Zanden et al. 2013).

For the above juvenile white shark case study, CSIA could confirm if variation in stable isotope values are caused by individuals either feeding on different prey in the same location or if individuals inhabit different regions, but feed on similar prey types. If a combined bulk stable isotope and CSIA approach identified that these animals inhabited different regions, i.e. multiple nursery grounds, this would have important consequences

for regional management (Figure 4.2). While white sharks are protected in South Africa, no legal protective framework exists in other West Indian Ocean countries and sharks are considered to be over-exploited by fisheries in the region (Smale 2008). During the newborn/juvenile life-stage, white sharks are not easily distinguishable from other species, thus it is possible that it is unknowingly part of fisheries catches. On the contrary, if CSIA revealed juveniles were foraging at different trophic levels in the same location, this would indicate a single nursery for this population, simplifying management efforts.

Telemetry to remotely monitor the movements of animals can also be combined with stable isotope data. To date, these methods are both improving confidence in isotope data interpretation and proposed management actions (Cunjak et al. 2005, Ceriani et al. 2012, Seminoff et al. 2012), but are also revealing dichotomies between movement patterns and previous inferences from stable isotope data. For example, stable isotope profiles of multiple tissues from individual bull sharks (*Carcharhinus leucas*) inhabiting a riverine/estuarine environment indicated individuals displayed a high degree of dietary specialization over time (Matich et al. 2011). Recently, through combining stable isotope analysis with telemetry, it was found that these sharks became more generalized on a seasonal basis to take advantage of prey pulses (Matich and Heithaus 2014). Stable isotope analysis of multiple tissues of a highly threatened leatherback turtle population off French Guiana also revealed two distinct foraging groups that agreed with previous satellite tracking data (Caut et al. 2008).

For juvenile white sharks, both acoustic and satellite telemetry data could reveal periods of residency, geographical areas of residency, and scales and timing of

movements. These data would confirm if the observed isotopic trends relate to the use of multiple nursery grounds, the physiological effect of some individuals undertaking large-scale movements compared to more resident individuals, and individual and population level feeding ecology characteristics (e.g., specialists vs. generalists) (Figure 4.2). These data would also confirm if in fact the variation in stable isotope values relate to the movement and habitats occupied by juveniles or if they were maternally inherited. If the latter were the case, the first isotope data following birth would allow unique insights in to the foraging location and trophic position of pregnant female white sharks prior to parturition (Figure 4.2). For global white shark populations, knowledge of gestation and parturition of females is extremely limited given only a few pregnant individuals have been caught and dissected (Francis 1996, Uchida et al. 1996, Christiansen et al. 2014b). The temporal trend of each individual, however would only allow estimation of the dilution of the maternal isotope values and incorporation rate of the juvenile's diet, with limited management impact (Figure 4.2).

Combining genetic analysis with stable isotope analysis is also leading to improved insights regarding animal migration patterns and population connectivity. Populations of Wilson's warblers (*Wilsonia pusilla*) on the North American east and west coasts were determined to be significantly different based on microsatellite DNA (Clegg et al. 2003). By combining this result with hydrogen stable isotope values, breeding latitude was estimated, allowing for the description of population connectivity between breeding and overwintering sites. For adult female southern right whales (*Eubalena australis*) sampled at a nursery ground, genetically related individuals had similar $\delta^{15}N$

and $\delta^{13}C$ isotope values indicating maternally inherited site fidelity to summer feeding grounds (Valenzuela et al. 2009).

White sharks in Australia have been shown to exhibit philopatric behavior (Pardini et al. 2001, Blower et al. 2012), with sporadic transoceanic dispersal events (Blower et al. 2012). Equally, juvenile sharks have been documented using multiple nursery areas with relatively restricted home ranges (Bruce and Bradford 2012). These behaviors would indicate that if juvenile sharks off Southern Africa are using different nursery grounds, then these individuals might be genetically distinct. It would then be expected that these genetically distinct groups of juveniles would have unique isotope values reflecting their foraging locations. For this juvenile white shark case study, correlations between stable isotope values and genetic diversity would provide support for multiple nurseries and confirm philopatric behavior for this species with major implications for management (Figure 4.2).

CONCLUSION

In modern science, conveying a message to the broader scientific and management community is typically restricted by the number of words allowed by the publishing journal (i.e., word counts). Moreover, when analyzing and interpreting large complex ecological datasets, common in the data rich world of today's science, drawing a single explanation or conclusion is difficult and often impossible. Yet, offering multiple interpretations is now viewed as speculative or the result of poor experimental design and has become frowned upon by a majority of scientific journal reviewers. Consequently, researchers are commonly forced in to selecting a single explanation to best describe the trends observed in their data. However, ignoring or avoiding alternative explanations of a

dataset to avoid negative reviews can not only undermine the overall message, but also hinder the scientific process. Ultimately, this can limit the development of effective management and conservation plans, particularly those that are adaptive. This case study on juvenile white shark vertebral stable isotope profiles revealed that multiple interpretations could be provided that equally explain the observed isotopic trends. One of these could be preferentially highlighted dependent on the motive of the study or researcher bias, but this could be either to the benefit or detriment of future white shark research and management actions. In this instance, it could be argued that additional research should be carried out prior to publishing data, but in these pressing environmental times, decisions are required based on the best current information available. We promote the use of stable isotopes incorporated in a multidisciplinary framework to improve our confidence in interpreting data, particularly when outcomes may inform conservation and management actions. If this is not possible, quality stable isotope data sets, with appropriate statistical analyses and potential alternative explanations should be clearly presented.

REFERENCES

Alonso MK, Crespo EA, García NA, Pedraza SN, Mariottie PA et al. (2002) Fishery and ontogenetic driven changes in the diet of the spiny dogfish, *Squalus acanthias*, in Patagonian waters, Argentina. Environ Biol Fish 63: 193-202

Aurioles D, Koch PL, Le Boeuf BJ (2006) Differences in foraging location of Mexican and California elephant seals: Evidence from stable isotopes in pups. Mar Mammal Sci 22: 326-338

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124: 83-494

Blower DC, Pandolfi JM, Bruce BD, Gomez-Cabrera MDC, Ovenden JR (2012) Population genetics of Australian white sharks reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. Mar Ecol Prog Ser 455: 229-244

Bolnick DI, Yang LH, Fordyce JA, Davis JM, Svanbäck R (2002) Measuring individual-level resource specialization. Ecology 83: 2936-2941

Bolnick DI, Svanbäck R, Fordyce JA, Yang LH, Davis JM et al. (2003) The ecology of individuals: incidence and implications of individual specialization. Am Nat 161: 1-28

Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol Appl 21: 1017-1023

Bonfil R, Meÿer M, Scholl MC, Johnson R, O'Brien S et al. (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310: 100-103

Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. Fems Microbiol Ecol 40: 85-95

Bruce BD, Bradford RW (2008) Spatial dynamics and habitat preferences of juvenile white sharks—identifying critical habitat and options for monitoring recruitment. Final report to Department of Environment, Water, Heritage and the Arts. Hobart, Australia: CSIRO

Bruce BD, Bradford RW (2012) Habitat use and spatial dynamics of juvenile white sharks, *Carcharodon carcharias*, in Eastern Australia. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 225-253

Campana SE, Natanson LJ, Myklevoll S (2002) Bomb dating and age determination of large pelagic sharks. Can J Fish Aquat Sci 59: 450-455

Carlisle AB, Kim SL, Semmens BX, Madigan DJ, Jorgensen SJ et al. (2012) Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). PLoS ONE 7: e30492

Carlisle AB, Goldman KJ, Litvin SY, Madigan DJ, Bigman JS et al. (2014) Stable isotope analysis of vertebrae reveals ontogenetic changes in habitat in an endothermic pelagic shark. Pro Roy Soc B 282: 20141446

Caut S, Guirlet E, Angulo E, Das K, Girondot M (2008) Isotope analysis reveals foraging area dichotomy for Atlantic leatherback turtles. PLoS ONE 3: e1845

Ceriani SA, Roth JD, Evans DR, Weishampel JF, Ehrhart LM (2012) Inferring foraging

areas of nesting loggerhead turtles using satellite telemetry and stable isotopes. PLoS ONE 7: e45335
Chapple TK, Jorgensen SJ, Anderson SD, Kanive PE, Klimley AP et al. (2011) A first estimate of white shark, *Carcharodon carcharias*, abundance off Central California. Biol Lett 7: 581-583
Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Mar Ecol Prog Ser 329: 281-287
Cherel Y, Hobson KA, Bailleul F, Groscolas R (2005) Nutrition, physiology and stable isotopes: new information from fasting and molting penguins. Ecology 86: 2881-2888
Christiansen HM, Hussey NE, Wintner SP, Cliff G, Dudley SFJ et al. (2014a) Effect of sample preparation techniques for carbon and nitrogen stable isotope analysis of hydroxyapatite structures in the form of elasmobranch vertebral central. Rapid Commun Mass Spectrom 28: 448-456
Christiansen HM, Lin V, Tanaka S, Velikanov A, Mollet HF et al. (2014b) The last frontier: catch records of white sharks (*Carcharodon carcharias*) in the northwest Pacific Ocean. PLoS ONE 9: e94407
Choy CA, Davison PC, Drazen JC, Flynn A, Gier EJ et al. (2012) Global trophic position comparison of two dominant mesopelagic fish families (Myctophidae, Stomiidae) using amino acid nitrogen isotopic analyses. PLoS ONE 7: e50133
Clegg SM, Kelly JF, Kimura M, Smith TB (2003) Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's warbler (*Wilsonia pusilla*). Mol Ecol 12: 819-830
Cliff G, Compagno LJV, Smale MJ, van der Elst RP, Wintner SP (2000) First records of white sharks, *Carcharodon carcharias*, from Mauritius, Zanzibar, Madagascar and Kenya. S Afr J Sci 96: 365-367
Couturier LIE, Rohner CA, Richardson AJ, Marshall AD, Jaine FRA et al. (2013) Stable isotope and signature fatty acid analyses suggest reef manta rays feed on demersal zooplankton. PLoS ONE 8: e77152
Cunjak RA, Roussel JM, Gray MA, Dietrich JP, Cartwright DF et al. (2005) Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. Oecologia 144: 636-646
DeNiro MJ, Epstein S (1978) Influence of diet of the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495-506
DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341-351
Domeier ML, Nasby-Lucas N (2013) Two-year migration of adult female white sharks (C*archarodon carcharias*) reveals widely separated nursery areas and conservation concerns. Anim Biotelem 1: 2-9
Estrada JA, Lutcavage M, Thorrold SR (2005) Diet and trophic position of Atlantic Bluefin tuna (*Thunnus thynnus*) inferred from stable carbon and nitrogen isotope analysis. Mar Biol 147: 37-45
France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124: 307-312

Francis MP (1996) Observations on a pregnant white shark with a review of reproductive biology. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 157-172
Gannes LZ, O'Brien DM, Martinez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats and a call for more laboratory experiments. Ecology 78: 1271-1276
Gannes LZ, Martínez del Rio C, Koch P (1998) Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comp Biochem Phys A119: 725-737
Heithaus MR, Vaudo JJ, Kreicker S, Layman CA, Krützen M et al. (2013) Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. Mar Ecol Prog Ser 481: 225-237
Herman DP, Burrow DG, Wade PR, Durban JW, Matkin CO et al. (2005) Feeding ecology of eastern North Pacific killer whales *Orcinus orca* from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Mar Ecol Prog Ser 302: 275-291
Hilderbrand GV, Farley SD, Robbins CT, Hanley TA, Titus K et al. (1996) Use of stable isotopes to determine diets of living and extinct bears. Can J Zool 74: 2080-2088
Hill JM, McQuaid CD, Kaehler S (2006) Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of southern Africa. Mar Ecol Prog Ser 318: 63-73
Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314-326
Hobson KA, Clark RG (1993) Turnover of ^{13}C in cellular and plasma fractions of blood: implications for non-destructive sampling in avian dietary studies. Auk 110: 638-641
Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84: 9-18
Hobson KA, Alisauskas RT, Clark RG (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. Condor 95: 388-394
Honda K, Kagiwada, H. Takahashi N, Miyashita K (2012) Seasonal stream habitat of adult Sakhalin taimen, *Parahucho perryi*, in the Bekanbeushi River system, eastern Hokkaido, Japan. Ecol Freshw Fish 21: 640-657
Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE et al. (2012) Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. Oecologia 169: 395-406
Hussey NE, Dudley SFJ, McCarthy ID, Cliff G, Fisk AT (2011) Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? Can J Fish Aquat Sci 68: 2029-2045
Hussey NE, McCann HM, Cliff G, Dudley SFJ, Wintner SP et al. (2012) Size-based analysis of diet and trophic position of the white shark (*Carcharodon carcharias*) in South African waters. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp 27-49
Hussey NE, MacNeil MA, McMeans B, Olin J, Dudley SFJ et al. (2014) Rescaling the trophic structure of marine food webs. Ecol Lett 17: 239-250

Hussey NE, MacNeil MA, Siple MC, Popp BN, Dudley SFJ et al. (2015) Expanded trophic complexity among large sharks. Food Webs 4: 1-7
Johnson SP, Schindler DE (2009) Trophic ecology of Pacific salmon (*Oncorhynchus* spp.) in the ocean: a synthesis of stable isotope research. Ecol Res 24: 855–863
Kharlamenko VI, Kiyashko SI, Imbs AB, Vyshkvartzev DI (2001) Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Mar Ecol Prog Ser 220: 103-117.
Kim SL, Tinker MT, Estes JA, Koch PL (2012) Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white sharks based on stable isotope analysis. PLoS ONE 7: e45068
Kiszka JJ, Aubail A, Hussey NE, Heithaus MR, Caurant F et al. (2015) Plasticity of trophic interactions among sharks from the oceanic south-western Indian Ocean revealed by stable isotope and mercury analyses. Deep-Sea Res Pt 1 96: 49-58
Lowe CG, Wetherbee BM, Crow GL, Tester AL (1996) Ontogenetic dietary shifts and feeding behavior of the tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. Environ Biol Fish 47: 203-211
Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biol Rev 84: 91-111
Matich P, Heithaus MR (2014) Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. J Anim Ecol 83: 199-213
Matich P, Heithaus MR, Layman CA (2010) Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). Can J Fish Aquat Sci 67: 877-885
Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J Anim Ecol 80: 294-305
McClelland JW, Montoya JP (2002) Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. Ecology 83: 2173-2180
McMeans BC, Olin JA, Benz GW (2009) Stable-isotope comparisons between embryos and mothers of a placentatrophic shark species. J Fish Biol 75: 2464-2474
Ménard F, Lorrain A, Potier M, Marsac F (2007) Isotopic evidence of distinct feeding ecologies and movement patterns in two migratory predators (yellowfin tuna and swordfish) of the western Indian Ocean. Mar Biol 153: 141-152
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48: 1135-1140
Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing models. Ecol Lett 11: 470-480
Navarro J, López L, Coll M, Barría C, Sáez-Liante R (2014) Short- and long-term importance of small sharks in the diet of the rare deep-sea shark *Dalatias licha*. Mar Biol 161: 1697-1707

Navarro J, Louzao M, Igual JM, Oro D, Delgado A et al. (2009) Seasonal changes in the diet of a critically endangered seabird and the importance of trawling discards. Mar Biol 156: 2571-2578
Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K et al. (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90: 961-974
Olin JA, Hussey NE, Fritts M, Heupel MR, Simpfendorfer CA et al. (2011) Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. Rapid Commun Mass Spectrom 25: 1008-1016
Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H et al. (2001) Sex-biased dispersal of great white sharks. Nature 412: 139-140
Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5: e9672
Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320
Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. Oecologia 136: 261-269
Phillips RA, Bearhop S, Mcgill RAR, Dawson DA (2009) Stable isotopes reveal individual variation in migration strategies and habitat preferences in a suite of seabirds during the nonbreeding period. Oecologia 160: 795-806
Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW et al. (2014) Best practices for use of stable isotope mixing models in food web studies. Can J Zool 92: 823-835
Ramos R, Ramírez F, Carrasco JL, Jover L (2011) Insights into the spatiotemporal component of feeding ecology: an isotopic approach for conservation management sciences. Divers Distrib 17: 338-349
Rosenblatt AE, Nifong JC, Heithaus MR, Mazzotti FJ, Cherkiss MS et al. (2015) Factors affecting individual foraging specialization and temporal diet stability across the range of a large "generalist" apex predator. Oecologia 178: 5-16
Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol Evol 19: 256-263
Seminoff JA, Benson SR, Arthur KE, Eguchi T, Dutton PH et al. (2012) Stable isotope tracking of endangered sea turtles: validation with satellite telemetry and $\delta^{15}N$ analysis of amino acids. PLoS ONE 7: e37403
Smale MJ (2008) Pelagic shark fisheries in the Indian Ocean. In: M.D. Camhi, E.K. Pikitch, E.A. Babcock (eds) Sharks of the Open Ocean. Blackwell Publishing, Oxford, pp 247-259
Trueman CN, McGill RAR, Guyard PH (2005) The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (*Salmo salar*). Rapid Commun Mass Spectrom 19: 3239-3247
Uchida S, Todo M, Teshima K, Yano K (1996) Pregnant white sharks and full-term embryos from Japan. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 139-155
Valenzuela LO, Sironi M, Rowntree VJ, Seger J (2009) Isotopic and genetic evidence for culturally inherited site fidelity to feeding grounds in southern right whales (*Eubalaena australis*). Mol Ecol 18: 782-791

Vander Zanden HB, Bjorndal KA, Reich KJ, Bolten AB (2010) Individual specialists in a generalist population: results from a long-term stable isotope series. Biol Lett 6: 711-714
Vander Zanden HB, Arthur KE, Bolten AB, Popp BN, Lagueux CJ et al. (2013) Trophic ecology of a green turtle breeding population. Mar Ecol Prog Ser 476: 237-249
Vaudo JJ, Matich P, Heithaus MR (2010) Mother-offspring isotope fractionation in two species of placentatrophic sharks. J Fish Biol 77: 1724-1727
Werry JM, Lee SY, Otway NM, Hu Y, Sumpton (2011) A multi-faceted approach for quantifying the estuarine-nearshore transition in the life cycle of the bull shark, *Carcharhinus leucas*. Mar Freshw Res 62: 1421-1431
Williams CT, Buck CL, Sears J, Kitaysky AS (2007) Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. Oecologia 153: 11-18
Wolf N, Carleton SA, Martínez del Rio C (2009) Ten years of experimental animal isotopic ecology. Funct Ecol 23: 17-26

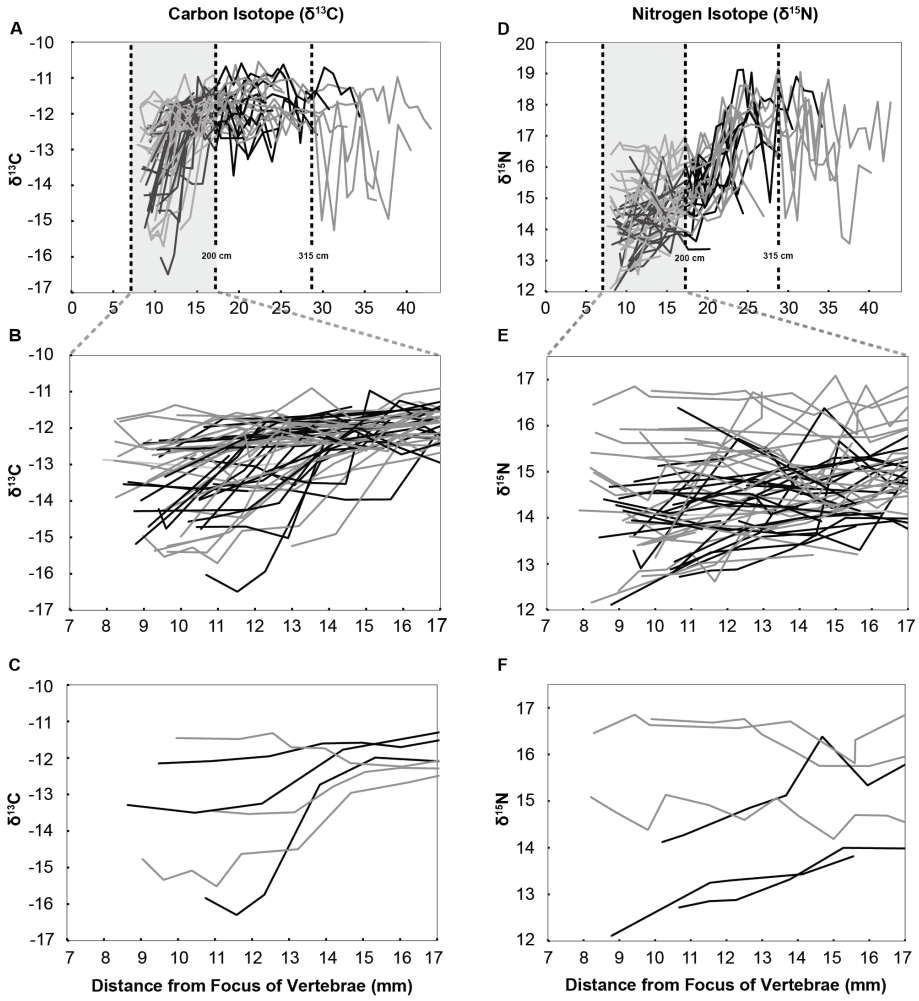

Figure 4.1 Stable isotope profiles of 58 white sharks from southern Africa. A single line represents each individual sampled approximately every 1–2 mm starting after the angle change (birth); black lines represent males, grey lines represent females. Distance is measured from the center of the vertebra (focus). **A**- Ontogenetic $\delta^{13}C$ values for each individual, dotted lines indicate trend changes in isotope values at approximately 200 cm precaudal length (PCL) and 315 cm PCL; **B**- Enlarged shaded area from A, $\delta^{13}C$ values for the juvenile portion, less than 17 mm from the focus (~200 cm PCL); **C**- $\delta^{13}C$ values from 6 individuals showing consistency in isotope values across sequential sampling points until convergence at approximately 17 mm from the focus; **D**- Ontogenetic $\delta^{15}N$ values for each individual, dotted lines indicate trend changes in isotope values at approximately 200 cm precaudal length (PCL) and 315 cm PCL; **E**- Enlarged shaded area from D, $\delta^{15}N$ values for the juvenile portion, less than 17 mm from the focus (~ 200 cm PCL); **F**- $\delta^{15}N$ values from 6 individuals showing the relative consistency in isotope values across sequential sampling points until convergence at approximately 17 mm from the focus.

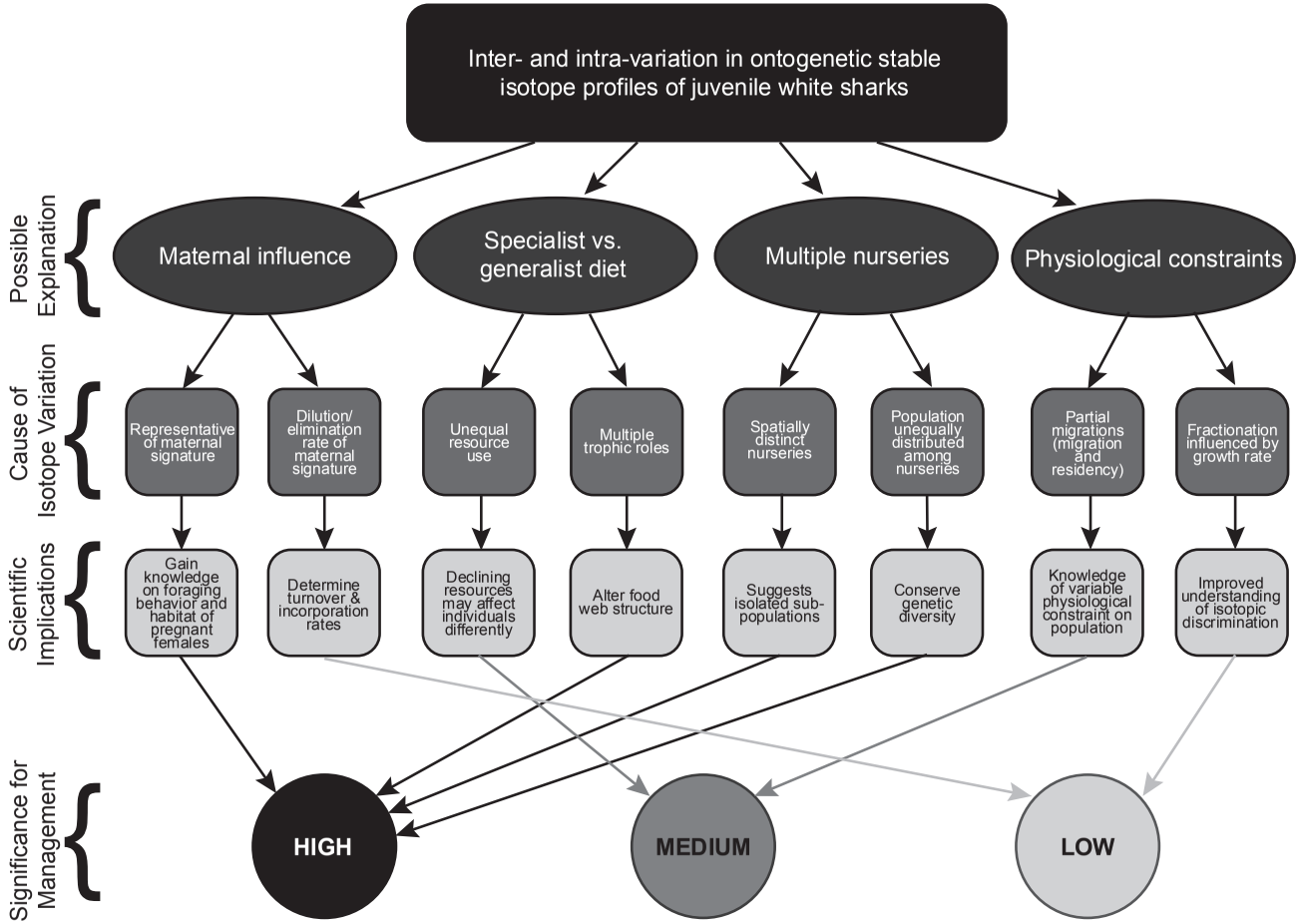

Figure 4.2 Viable explanations of variation in stable isotope values across the vertebrae of juvenile white sharks from southern Africa and their management implications. Specific causes of each explanation are listed along with their scientific implications and significance to management (designated as High, Medium or Low impact).

CHAPTER 5: EXAMINING HABITAT USE AND MOVEMENTS WITHIN AND AMONG SUB-ADULT WHITE SHARKS (*CARCHARODON CARCHARIAS*) OFF SOUTHERN AFRICA

INTRODUCTION

Determining spatial connectivity in widely distributed migratory marine animals is critical for conservation (Rubenstein and Hobson 2004, Martin et al. 2007, Wallace et al. 2010), however long life spans and inaccessibility to the species across most of their range make it difficult to determine these characteristics. Often, to create conservation and management plans generalizations about habitat use and migration are made at the species level, assuming that all individuals undertake the same strategy without accounting for changes in behavior due to life-stage, sex, or size (Hammerschlag-Peyer and Layman 2010). While creating management plans at the species level is useful, especially for populations with limited data, the importance of individual specialization to foraging behavior (Araújo et al. 2009, Newsome et al. 2009, Hückstädt et al. 2012) and habitat use (Hammerschlag-Peyer and Layman 2010) has been recognized. The degree to which individuals use a resource has important implications for population stability in the face of changing resources or prey availability.

The white shark, *Carcharodon carcharias*, is a large migratory predator that is distributed throughout the world's temperate and tropical oceans (Compagno 2001) and is listed as vulnerable on the IUCN Red List (IUCN 2015). Knowledge of key characteristics such as habitat use, movement, and migration patterns vary regionally (Domeier 2012, Dudley 2012). There is a well-documented aggregation in the southwest Indian Ocean along the coast of South Africa (Dudley 2012, see references therein) where precautionary protection was extended to in 1991 due to the poorly known details on the white shark's ecological and life-history traits (Compagno 1991). The majority of

research on white sharks in South Africa has been based on individuals captured in the beach protection nets along KwaZulu-Natal (KZN) or focused on animals associated with cape fur seal (*Arctocephalus pusillus pusillus*) colonies in the Western Cape (Dudley 2012), which may have skewed the perception of this population's characteristics. It was originally thought that the center of white shark distribution was the Western Cape with individuals extending north to southern Mozambique (Bass et al. 1975). As well, a preliminary examination of white shark populations along the coast of southern Africa has shown size segregation in white shark habitat use; smaller individuals with a mode fork length (FL) of 230-234 cm FL (females) and 241-245 cm FL (males) were captured in bather protection nets in KZN (mean= 219 cm) (Cliff et al. 1989). Whereas, larger sharks are typically reported from the Western Cape; Ferreira and Ferreira (1996) found the most common size of white sharks were in the 227-262 cm FL and 270-307 cm FL size ranges (mean=280). Kock and Johnson (2006) also reported that white sharks in Mossel Bay and Gansbaai (242-285 cm FL) and False Bay (286-329 cm FL) were larger than those in KZN.

Tag-recapture data has shown white sharks from South Africa undertaking long-distance coastal movements (774 km and 1409 km) between KZN and the Eastern and Western Cape (Cliff et al. 1996a). White sharks are documented to begin incorporating marine mammals in their diet at ~217 cm FL (Hussey et al. 2012) therefore, it is believed that juveniles and small sub-adults are primarily located in the KZN and then begin seasonal migrations to the Western Cape to take advantage of resources available at the seal colonies. Bonfil et al. (2005) used satellite and acoustic telemetry on individuals ranging in size from 218-300 cm FL in the Western Cape and described four movement

strategies including; long-distance oceanic migrations, coastal migrations, smaller scale patrolling and residency. Residency and sexual segregation was described for white sharks in False Bay where, females were present year round, while males were present during the winter only (Kock et al. 2013). The degree to which individuals adopt the above movement strategies is currently unknown for this population. As white sharks are protected in the waters of South Africa, but not along the east coast of Africa or offshore, it is important to determine how this population is distributed and its movement patterns within this region.

Stable isotopes have increasingly been used to investigate the spatial and foraging ecology of a species or populations (Hobson 1999, Boecklen et al. 2011, McMahon et al. 2013). The ratio of nitrogen stable isotopes ($\delta^{15}N$) increase in a predictable way between predator and prey and is typically used as an indicator of relative trophic level (DeNiro and Epstein 1981, Minagawa and Wada 1984, Post 2002, Hussey et al. 2014). The ratio of carbon stable isotopes ($\delta^{13}C$) is more conservative between predator and prey but reflects the sources of primary production (e.g., benthic vs. pelagic). Consequently carbon stable isotopes can be used to identify where an individual has foraged (DeNiro and Epstein 1978). A gradient in $\delta^{13}C$ values along the South African coastline has been described such that in nearshore waters $\delta^{13}C$ values increase moving from northeast (KZN) to southwest (Cape) and the nearshore environment is more enriched in ^{13}C than offshore, pelagic waters (Hill et al. 2006); thus creating distinct isotope values for different bioregions along the South African coast, that provide a method to estimate habitat use for white sharks in this region (Best and Schell 1996, Carlisle et al. 2012, Carlisle et al. 2012).

Accretionary structures such as vibrasse, hair, scute and elasmobranch vertebrae are metabolically stable, recording the stable isotope values at time of formation and reflecting an individual's diet and environment throughout its life. Through serial sampling of accretionary structures, ontogenetic trends in isotope values have provided important information on changes in habitat and foraging in southern right whales *(Eubalaena australis*; Best and Schell 1996), sea otters (*Enhydra lutris nereis;* Newsome et al. 2009), salmon sharks (*Lamna ditropis*; Carlisle et al. 2014), and populations of white sharks in the northwest Atlantic Ocean (Estrada et al. 2006) and northeast Pacific Ocean (Kim et al. 2012, Carlisle et al. 2012). As well, individual dietary specialization can be investigated through ontogenetic sampling of accretionary structures and stable isotope analysis (Newsome et al. 2009, Hückstädt et al. 2012), providing details on how resource use varies within and among individuals of a population.

The aim of this study was to estimate habitat use in sub-adult white sharks from southern Africa. Given preliminary observations on movement in white sharks from telemetry data, I predict individuals will obtain the majority of their resources from the Cape region, but several movement strategies will exist within the population. To investigate patterns at the population level, ontogenetic profiles of $\delta^{15}N$ and $\delta^{13}C$ in the vertebrae were quantified and incorporated into a Bayesian mixing model to determine regional contribution to the diet of white sharks. The variation within and among individuals was then investigated to elucidate changes in habitat use within the population during this life-stage.

METHODS

Sample collection and preparation

White sharks (n=98, 59 females and 39 males) in this study were incidentally captured in the beach protection nets off KwaZulu-Natal, South Africa between the years 1984 to 2012 (Cliff and Dudley 2011). Additional vertebral centra were obtained from one female captured off Gansbaai in the Western Cape (1987) and from one female captured off the coast of Malindi, Kenya (1996). The sex and precaudal length were recorded for all sharks, with precaudal length (PCL) measured as the straight-line distance from the tip of the snout to the precaudal notch as defined by Dudley et al. (2005), then converted to straight fork length (FL) using the following equation: FL=1.100 PCL + 3.554 (n=142) (Cliff et al. 1996b). Vertebral centra were excised from anterior to the first dorsal fin and stored frozen. Prior to analysis vertebrae were cleansed of excess tissue and dried at 40°C for 48 hours. The vertebral radius (VR) was recorded and a bow-tie section (5 mm) was cut from each vertebral centrum using an IsoMet® low speed diamond saw (Buehler Canada, Whitby, ON, Canada). Using 0.5 mm round carbide bits (Brasseler Canada, Quebec, QC, Canada) vertebrae were serially sampled from the outer-most portion of the corpus calcareum towards the focus of the vertebrae (Figure 5.1). Distance from the focus of the vertebrae to the center of each sampling location was measured.

To define the sub-adult portion of the vertebral centra the relationship between VR and FL was determined (Figure 5.2). This relationship was best defined by a linear regression and allowed for an estimation of FL at a specific VR. The lower size limit for sub-adults was defined as the point at which white sharks begin to incorporate marine mammals into their diets (194 cm PCL (217 FL) (Hussey et al. 2012)). The upper size limit for sub-adults was defined as 344 cm FL, which is the size at 50% maturity for male

white sharks captured in the KZN beach protection program (Dudley and Simpfendorfer 2006). Therefore, samples from 17 mm to 29 mm from the focus of the vertebrae were defined as the sub-adult region.

Stable isotope analysis

The number of samples per individual shark was dependent on the size of vertebral radius and ranged from 1 to 17 (5 ±3.5, mean ± standard deviation (SD)). An average of 811 μg of powdered vertebrae was weighed into tin capsules. Continuous flow isotope ratio mass spectrometry was performed on all samples using a Costech 4010 elemental analyzer (Costech Instruments, Valencia, CA, USA), Conflo IV gas interface (Thermo Scientific, Waltham, MA, USA) coupled to a Delta V™ Advantage mass spectrometer (Thermo Scientific, Waltham, MA, USA) at the Great Lakes Institute for Environmental Research, University of Windsor (Windsor, Canada). Stable isotope ratios are expressed in delta (δ) values as the ratio of an unknown sample to a recognized standard and are expressed in parts per thousand (or per mil, ‰) using the following equation:

$$\delta^{b}X = [(R_{sample}/R_{standard})-1] \times 1000 \quad (1)$$

where X is the element, b is the mass of the heavy isotope (less abundant) and R_{sample} and $R_{standard}$ are the heavy to light isotope ratio (for example; carbon, $^{13}C/^{12}C$, nitrogen: $^{15}N/^{14}N$) of the sample and standard, respectively (Peterson and Fry 1987). To measure analytical precision the standard deviation of replicate analyses for four standards; National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA) 8414 (n=142, up to 2014), NIST1577c (n=132, since 2014), internal lab standard (tilapia muscle, n=204), USGS 41 (n=67, since 2015) were used. The standard deviation was

≤0.2‰ for $\delta^{15}N$ and ≤0.16‰ for $\delta^{13}C$ for all the standards. The accuracy, based on the certified values of USGS 40 (n=62, since 2015) analyzed throughout runs showed a difference of 0.05‰ for $\delta^{15}N$ and -0.02‰ for $\delta^{13}C$ from the certified value. Instrumentation accuracy checked throughout the period of time that these samples were analyzed was based on NIST standards 8573, 8548, and 8547 for $\delta^{15}N$ and 8542 and 8573, for $\delta^{13}C$ (n=61 for all). The mean differences from the certified values were 0.05, 0.1, 0.03‰ for $\delta^{15}N$ and 0.04 and 0.01‰ for $\delta^{13}C$ respectively. Due to the length of time over which samples were run lab standards have changed reflected in the differing totals for standard runs.

Prey data

Published (Daly et al. 2013, Hussey et al. 2015, Kiszka et al. 2015) and unpublished $\delta^{13}C$ and $\delta^{15}N$ values of known white shark prey (from stomach content analysis, Hussey et al. 2012) and potential prey species (i.e., not documented in stomach content analysis, but co-occurring in the same region as white sharks) were collated. Stable isotope data of prey items of white sharks in the southwest Indian Ocean were divided into three bioregions (Figure 5.3) to define the isotope values for each region including; Kwazulu-Natal and tropical waters (including north to Mozambique and Madagascar) (hereafter referred to as KZN), Pelagic (all pelagic waters in the region), and temperate waters of the Eastern and Western Cape (hereafter referred to as Cape). Prey species were grouped into one of the three regions based on their primary distribution (Table S5.1). Species that are known to occur in two or more regions were excluded from the analysis (n=27). For species where stable isotope values were available for multiple individuals, individual values were resampled 2000 times from

each $\delta^{13}C$ and $\delta^{15}N$ distribution to create a mean ± SD for that species (Table S5.1). As species with more individuals sampled were likely to be more representative of the species isotope value, a weighted mean ± SD of all species in a region was calculated for each region and for both stable isotopes.

Statistical analysis

Due to the time frame over which samples were collected (1984-2012) a linear regression was used to examine the relationship between the terminal sample removed from the outer most portion of the vertebrae (i.e., formed during year of capture) and capture year to investigate if there was a significant effect of year of capture on isotope values. The VR of 12 individuals extended past the sub-adult phase and therefore their terminal sample was not included in this analysis.

A Bayesian stable isotope-mixing model, MixSIAR GUI (Semmens et al. 2009, Parnell et al. 2013, Stock and Semmens 2015) was used to estimate the relative contribution of prey groups from each of the isotopically distinct bioregions to the diet of white sharks. It was assumed that resource use in a region is proportional to time spent in the region, therefore the relative contribution of prey items to an individual was used a proxy for habitat use. It is accepted that individuals could be spending a portion of their time in regions where they do not obtain any resources. Using MixSIAR the probability distribution of each potential prey item (i.e., region) to a consumer's stable isotope values is estimated, while accounting for variability among individuals and uncertainty associated with diet-tissue discrimination factors (Phillips et al. 2014). The diet-tissue discrimination factor for vertebral cartilage for $\delta^{13}C$ (3.75 ± 0.44) and $\delta^{15}N$ (1.45 ± 0.61) derived for large sharks (Hussey et al. 2010) was included in the model to adjust prey

item values. The model was run with individual vertebral samples nested within individual sharks as a random effect and uninformative priors were used. A hierarchical cluster analysis was then conducted on the 50th percentile probability distributions obtained from MixSIAR, to group individual white sharks with similar regional prey contributions.

Mixed-model variance component analysis was used to examine the observed variability of white shark $\delta^{13}C$ and $\delta^{15}N$ values (each isotope modeled separately). The data was modeled for all individuals combined with individual as the random component and sex as a fixed effect. To investigate variation in habitat use within bioregion, models were run for the four groups of white sharks clustered by median prey contribution with individual and region as random effects and sex as a fixed effect. The intercept variability represents the variability between individuals (between-individual component (BIC)) and the residual error term represents the variability within an individual (within-individual component (WIC)) (Newsome et al. 2009). The total observed variability (total isotopic niche width (TNW)) was calculated as the sum of the BIC and WIC (Newsome et al. 2009). The degree of specialization was calculated by the WIC/TNW ratio, whereas values approaching 0 indicated individuals are specialists and values approaching 1 indicate individuals are more generalists (Hückstädt et al. 2012). All analyses were performed in the R computing environment (R Core Team 2016).

RESULTS

Isotopic results of all vertebrae samples (n=498, Figure 5.4A, B) indicate there was large variation in both the $\delta^{13}C$ (-16.21‰ to -9.95‰) and $\delta^{15}N$ (13.41‰ to 19.46‰) across sub-adult white sharks sampled in South Africa. There was no relationship

between vertebral $\delta^{13}C$ and distance from focus (linear regression n=498, p=0.16, r^2=0.004) (Figure 5.4A) or with year (linear regression n=88, p=0.28, r^2=0.01) (Figure 5.5A). There was a significant positive relationship between vertebral $\delta^{15}N$ and distance from focus (linear regression, n=498, p <0.001, r^2= 0.26) (Figure 5.4B), and a significant negative relationship between vertebral $\delta^{15}N$ and year (linear regression n=88, p=0.04, r^2=0.05) (Figure 5.5B), but the low r^2 value for the sample year versus $\delta^{15}N$ relationship would suggest this was a minor factor.

Stable isotope data for 75 prey species were grouped by region of occurrence (Table S5.1) and each region had distinct $\delta^{13}C$ and $\delta^{15}N$ values (Figure 5.5). The Cape region was the most enriched in ^{13}C (-14.6‰ ± 0.7, mean ± SD) and ^{15}N (16.3‰ ± 0.8), $\delta^{15}N$ values for the pelagic and KZN regions were similar (13.0‰ ± 1.4 and 13.22‰ ± 2.0, respectively), however the pelagic region was more depleted in ^{13}C (-17.0‰ ± 0.6; -15.8‰ ± 0.9, pelagic and KZN, respectively) as would be expected. The majority of the white shark vertebral $\delta^{13}C$ and $\delta^{15}N$ values were between these mean regional prey isotope values (Figure 5.6).

The mixing model results showed white sharks varied in their use of each region with white sharks clustering into four groups based on median prey contribution (Figure 5.7). Overall, 19% of the sharks clustered into KZN, which had the highest median prey contribution from KZN prey (48%) and approximately equal median contributions from Pelagic (23%) and Cape (24%) (Figure 5.8A). Nearly a quarter (23%) of the sharks clustered into Pelagic, which had the highest median prey contribution from Pelagic prey (52%) with lower median contributions from KZN (14%) and Cape (31%) (Figure 5.8B). Half of the sharks had the highest median contributions from the Cape prey group

however, 23% clustered into Cape 1 which had a very high median contribution from Cape prey (73%) and lower contributions from KZN and Pelagic (12% each) (Figure 5.8C) and 35% of the sharks clustered into Cape 2 which had a lower, but still dominant prey contribution from the Cape (51%) and equal contributions from KZN and Pelagic (22% each) (Figure 5.8D).

The mixed-effect model variance component analysis for all individuals combined indicated that sex was not significant in explaining variance in $\delta^{13}C$ or $\delta^{15}N$ in white shark vertebrae (Table 5.1). The WIC/TNW was 0.60 suggesting white sharks off southern Africa are weakly generalist predators (Table 5.1). The mixed-effects model variance component analysis was run for each of the clustered groups individually to examine within strategy (region) individual specialization. Sex was a significant factor in explaining the variance in $\delta^{13}C$ and $\delta^{15}N$ for the group with the lower prey contribution from the Cape (Cape 2) and for $\delta^{15}N$ for the KZN dominant group, but not the other groups. All individuals grouped by region were classified as generalists however, when investigating the $\delta^{13}C$ and $\delta^{15}N$ specific WIC/TNW values, individuals within a group ranged from strong to weak generalists (Table 5.1).

DISCUSSION

Understanding the variation within and among individuals of a species in terms of habitat use, movement, and migration has important implications for conservation and management (Wallace et al. 2011, Goodman-Hall et al. 2015). Due to a known gradient in stable isotope values along the coast of southern Africa (Hill et al. 2006, Hill and McQuaid 2008) and based on the estimated regional prey contribution it was possible to investigate the spatial ecology of sub-adult white sharks in this region using $\delta^{13}C$ and

$\delta^{15}N$ in their vertebrae. Mixing model results identified that white sharks have four main strategies of habitat use; suggesting that sub-adult white sharks have a high degree of movement.

This study indicates that white sharks in South Africa are consuming prey from all regions to some degree and there were no individuals that were residential in just one region. There is an inherent bias to this data, as all but two sharks were obtained from bather protection nets in KZN therefore, if individual sharks were truly residential in the Cape region they would not be represented in this study. Although tagging data has provided valuable insight to the movement and residency patterns of white sharks off southern Africa (Cliff et al. 1996a, Bonfil et al. 2005, Kock et al. 2013, Towner et al. 2013), the extent to which white sharks off South Africa are residential or migratory off was unknown. The four groups identified by proportion of regional source prey contribution to white sharks provide details on individual strategies of habitat use and movement. As predicted there was a strong contribution of prey from the Cape region; where, prey contribution from the Cape region was strongest in 58% of the individuals in this study (Cape 1 and Cape 2) and accounted for at least a quarter of the prey contribution for the KZN and Pelagic prey dominated groups. The strong contribution of Cape prey to all individuals agrees with previous tagging data highlighting the importance of the Cape region to sub-adult white sharks (Cliff et al. 1996a, Bonfil et al. 2005, Kock et al. 2013, Towner et al. 2013).

White sharks are known to occur in the coastal waters of KZN, as evidenced by capture in the bather protection nets (Cliff et al. 1989, Cliff et al. 1996b), however little is known of sub-adults residency and movements within this region. One individual tagged

in KZN by Cliff et al. (1996a) was recaptured in the same location one year later, although this individual was a juvenile and its movements during time at liberty are unknown. Prey contribution from KZN was highest in 19% of the sharks suggesting that a portion of the white shark population is likely resident with KZN for at least portion of the year. Prey from the region classified as KZN also included species that are common in the tropical waters along the eastern coast of Africa. Large white sharks are documented to occur along the east coast of Africa and in the western Indian Ocean (Cliff et al. 2000, Zuffa et al. 2002) and this data indicates sub-adult white sharks are likely using this habitat also. For nearly a quarter of the population, the greatest prey contribution was obtained from pelagic resources. Indicating these individuals are potentially making offshore migrations similar to those documented by Bonfil et al. (2005) using satellite telemetry. The use of the Pelagic region and KZN by all white sharks in this study highlights the requirement for further management of white sharks outside of South African waters and the need for international cooperation to monitor this population.

Overall, white sharks in this study were classified as weak generalists agreeing with previous research based on stomach content analysis and muscle stable isotope analysis for white sharks in South Africa (Hussey et al. 2012). The degree of individual specialization observed within this population was likely due to dominant regional habitat use and movement strategies among individuals and the resources available in those regions. Individual specialization was described within a generalist population for white sharks from the northeast Pacific Ocean (Kim et al. 2012). However, extensive satellite tagging of white sharks in the northeast Pacific Ocean has identified a general migration

pattern undertaken with seasonal coastal aggregation followed by annual (male) or biannual (female) offshore migrations (Boustany et al. 2002, Jorgensen et al. 2010, Domeier 2012, Domeier and Nasby Lucas 2012, 2013). Therefore, the individual specialization observed in the Pacific population is likely representative of dietary specialization and not different migration strategies.

When accounting for the region an individual was clustered into (i.e., based on highest median prey contribution) the degree of individual specialization (WIC/TNW) for $\delta^{13}C$ varied among regional groups. Individuals classified as Cape 1 were the most generalized of the four regions. These individuals had the highest prey contribution from a single region for all groups; therefore it is likely they are spending the majority of their time in the Cape region. The high variation observed within individuals likely reflects time spent as residents in the Cape region versus time spent in other regions. The WIC/TNW value for $\delta^{15}N$ for this group also supports a dietary generalist pattern. The group (KZN) with the lowest $\delta^{13}C$ WIC/TNW value could not strictly be classified as specialist or generalist. Interestingly, the KZN group had the highest $\delta^{15}N$ WIC/TNW value indicating, while individuals had a wide prey base they were more specialized in their foraging locations. Cape 2 was the only group in which sex was a significant factor in explaining the variation in observed stable isotope values (both $\delta^{13}C$ and $\delta^{15}N$). Kock et al. (2013) detected both males and females around the seal colony in False Bay during autumn and winter. However, during summer it was determined that females shifted to an inshore habitat while males were not detected in the bay (Kock et al. 2013). The sexual segregation of individuals in this region would account for the observed variability in stable isotopes by sex. Although, sex did not explain a significant amount of the variation

for the Cape 1 group, indicating that while there is sexual segregation occurring in False Bay, it may not occur throughout the Eastern and Western Cape.

In this study, the relationship between $\delta^{15}N$ and distance from the focus was likely due to both an increase in trophic level and movement between regions as evidenced by the mixing model results. These data agree with previous stomach content analysis and $\delta^{15}N$ in muscle tissues of white sharks from South Africa, which also showed an increase in trophic position with size (Hussey et al. 2012). Increasing vertebral $\delta^{15}N$ over ontogeny has been reported in both the northwest Atlantic Ocean (Estrada et al. 2006) and northeast Pacific Ocean (Kim et al. 2012) and suggests an increase in trophic position with size. An increase in trophic position with body size is a common phenomena in fish, and is usually attributed to increasing mouth gape size and opportunity to prey on larger size prey (Romanuk et al. 2011). However, Carlisle et al. (2012) reported decreasing muscle $\delta^{15}N$ in white sharks with total length, which was attributed to time spent offshore foraging on prey with a lower $\delta^{15}N$, which may or may not have been at a lower trophic level. The decrease in $\delta^{15}N$ with sample year observed in this study may be an artifact of shark size at year of capture (i.e., smaller sharks with lower $\delta^{15}N$ were caught in more recent years) and not a true shift in $\delta^{15}N$ values in the environment. A general decrease in global $\delta^{13}C$ over the past ~100 years due to increasing anthropogenic release of the Earth's surface carbon reservoirs has also been reported (Long et al. 2005). However, this relationship was not observed in this study, possibly due to the relatively short time period animals were sampled ~40 years and the large variation in $\delta^{13}C$ among individuals.

These results provide valuable insight in to the habitat use of sub-adult white

sharks off southern Africa. Stable isotope analysis provides evidence that sub-adult white sharks are generalist predators that incorporate a wide range of prey and have a large spatial range. It also indicates that while white sharks typically have one dominant region where they obtain their prey, they are using resources from all regions investigated. Further research is required to examine the extent of residency in KZN and along the east coast of Africa. Management plans for white sharks off southern Africa should account for the variation observed in movement and migration among individuals. Specifically, international cooperation is needed to manage this population, as it is likely that white sharks spend a significant portion of their time in international waters.

REFERENCES

Araújo MS, Bolnick DI, Layman C (2011) The ecological causes of individual specialisation. Ecol Lett 14: 948–958

Bass AJ, D'Aubrey JD, Kistnnasamy N (1975) Sharks of the east coast of Southern Africa. 4. The families Odontaspididae, Scapanorhynchidae, Isuridae, Cetorhinidae, Alopiidae, Orectolobidae and Rhiniodontidae. Invest Rep Oceanogr Res Inst, Durban S Afr 39: 1-102

Best PB, Schell DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124: 483-494

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42: 411-440

Bonfil R, Meyer M, Scholl MC, Johnson R, O'Brien S et al. (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310: 100-103

Boustany AM, Davis SF, Pyle P, Anderson SD, Le Boeuf BJ et al. (2002) Expanded niche for white sharks. Nature 415: 35-36

Carlisle AB, Kim SL, Semmens BX, Madigan DJ, Jorgensen SJ et al. (2012) Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). PLoS ONE 7(2): e30492

Carlisle AB, Goldman KJ, Litvin SY, Madigan DJ, Bigman JS et al. (2014) Stable isotope analysis of vertebrae reveals ontogenetic changes in habitat in an endothermic pelagic shark. Proc R Soc B 282: 20141446

Cliff G, Dudley SFJ (2011) Reducing the environmental impact of shark-control programs: a case study from KwaZulu-Natal, South Africa. Mar Freshw Res 62: 700-709

Cliff G, Dudley SFJ, Davis B (1989) Sharks caught in the protective gill nets off Natal, South Africa: 2.The great white shark *Carcharodon carcharias* (Linnaeus). S Afr J Mar Sci 8: 131-144

Cliff G, van der Elst RP, Govender A, Witthuhn TK, Bullen EM (1996a) First estimates of mortality and population size of white sharks on the South African coast. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 393-400

Cliff G, Dudley SFJ, Jury MR (1996b) Catches of white sharks in KwaZulu-Natal, South Africa and environmental influences. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 351-362

Cliff G, Compagno LJV, Smale MJ, van der Elst RP, Wintner SP (2000) First records of white sharks, *Carcharodon carcharias*, from Mauritius, Zanzibar, Madagascar and Kenya. S Afr J Sci 96: 365-367

Compagno LJV (1991) Government protection for the great white shark (*Carcharodon carcharias*) in South Africa. S Afr J Sci 87: 284-285

Compagno LJV (2001) Sharks of the world: An annotated and illustrated catalogue of shark species known to date. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Cat Fish

Purposes No. 1 2: 1-269
Daly R, Froneman PW, Smale MJ (2013) Comparative feeding ecology of bull sharks (*Carcharhinus leucas*) in the coastal waters of the southwest Indian Ocean inferred from stable isotope analysis. PLoS ONE 8(10): e78229
DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42: 495-506
DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45: 341-351
Domeier ML (2012) A new life-history hypothesis for white sharks, *Carcharodon carcharias,* in the Northeastern Pacific. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 199-223
Domeier ML, Nasby-Lucas N (2012) Sex-specific migration patterns and sexual segregation of adult white sharks, *Carcharodon carcharias,* in the Northeastern Pacific. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 133-146
Domeier ML, Nasby-Lucas N (2013) Two-year migration of adult female white sharks (*Carcharodon carcharias*) reveals widely separated nursery areas and conservations concerns. Anim Biotelem 1: 1-10
Dudley SFJ (2012) A review of research on the white shark, *Carcharodon carcharias*, in Southern Africa. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 511-533
Dudley SFJ, Simpfendorfer CA (2006) Population status of 14 shark species caught in the protective gillnets off KwaZulu-Natal beaches, South Africa, 1978-2003. Mar Freshw Res 57: 225-240
Dudley SFJ, Cliff G, Zungu MP, Smale MJ (2005) Sharks caught in protective gill nets off KwaZulu-Natal, South Africa. 10. The dusky shark, *Carcharhinus obscurus* (Lesueur 1818). Afr J Mar Sci 17: 107-127
Estrada JA, Rice AN, Natanson LJ, Skomal GB (2006) Use of isotopic analysis of vertebrae in reconstructing ontogenetic feeing ecology in white sharks. Ecology 87: 829-834
Ferreira CA, Ferreira TP (1996) Population dynamics of white sharks in South Africa. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias.* Academic Press, San Diego, pp 381-391
Goodman Hall A, Avens L, Braun McNeill J, Wallace B, Goshe LR (2015) Inferring long-term foraging trends of individual juvenile loggerhead sea turtles using stable isotopes. Mar Ecol Prog Ser 537: 265-276
Hammerschlag-Peyer CM, Layman CA (2010) Intrapopulation variation in habitat use by two abundant coastal fish species. Mar Ecol Prog Ser 415: 211–220
Hill JM, McQuaid CD (2008) $\delta^{13}C$ and $\delta^{15}N$ biogeographic trends in rocky intertidal communities along the coast of South Africa: Evidence of strong environmental signatures. Estuar Coast Shelf S 80: 261–268.
Hill JM, McQuaid CD, Kaehler S (2006) Biogeographic and nearshore-offshore trends in isotope ratios of intertidal mussels and their food sources around the coast of Southern Africa. Mar Ecol Prog Ser 318: 63–73

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314–326
Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE et al. (2012) Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. Oecologia 169: 395–406
Hussey NE, Brush J, McCarthy ID, Fisk AT (2010) Diet-tissue discrimination factors for large sharks under semi-controlled conditions. Comp Biochem Physiol Part A 155: 445-453
Hussey NE, McCann HM, Cliff G, Dudley SFJ, Wintner SP et al. (2012) Size-based analysis of diet and trophic position of the white shark, *Carcharodon carcharias*, in South African waters. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp. 27-49
Hussey NE, MacNeil MA, McMeans BC, Olin JA, Dudley SFJ et al. (2014) Rescaling the trophic structure of marine food webs. Ecol Lett 17: 239-250
Hussey NE, MacNeil MA, Siple MC, Popp BN, Dudley SFJ et al. (2015) Expanded trophic complexity among large sharks. Food Webs 4: 1-7
IUCN 2015. *The IUCN Red List of Threatened Species. Version 2015-4*. <http://www.iucnredlist.org>. Downloaded on 2 February 2016.
Jorgensen SJ, Reeb CA, Chapple TA, Anderson S, Perle C et al. (2010) Philopatry and migration of Pacific white sharks. Proc R Soc B 277: 679-688
Kim SL, Tinker MT, Estes JA, Koch PL (2012) Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white sharks based on stable isotope analysis. PLoS ONE 7: e45068
Kiszka JJ, Aubail A, Hussey NE, Heithaus MR, Caurant F et al. (2015) Plasticity of trophic interactions among sharks from the oceanic south-western Indian Ocean revealed by stable isotope and mercury analyses. Deep Sea Res Pt I 96: 49-58
Kock A, Johnson R (2006) White shark abundance: Not a causative factor in numbers of shark bite incidents. In: Nel DC Peschak TP (eds) Finding a balance: White shark conservation and recreational safety in the inshore waters of Cape Town, South Africa. Proceedings of a specialist workshop. WWF South Africa Report Series-2006/Marine/001 Annexure 1 pp 1-19
Kock A, O'Riain MJ, Mauff K, Meÿer M, Kotze D et al. (2013) Residency, habitat use and sexual segregation of white sharks, *Carcharodon carcharias* in False Bay, South Africa. PLoS ONE 8(1): e55048
Long ES, Sweitzer RA, Diefenbach DR, Ben-David M (2005) Controlling for anthropogenically induced atmospheric variation in stable carbon isotope studies. Oecologia 146: 148-156
Martin TG, Chadès I, Arcese P, Marra PP, Possingham HP et al. (2007) Optimal conservation of migratory species. PLoS ONE 2: 3-7
McMahon KW, Ling Hamady L, Thorrold SR (2013) A review of ecogeochemistry approaches to estimating movements of marine animals. Limnol Oceanogr 58: 697-714
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmochim Acta 48: 1135-1140

Newsome SD, Tinker MT, Monson DH, Oftedal OT, Ralls K et al. (2009) Using stable isotopes to investigate individual diet specialization in California sea otters (*Enhydra lutris nereis*). Ecology 90: 961-974

Parnell AC, Phillips DL, Bearhop S, Semmens BX, Ward EJ et al. (2013) Bayesian stable isotope mixing models. Environmetrics 24: 387-399

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18: 293-320

Phillips DL, Inger R, Bearhop S, Jackson AL et al. (2014) Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92: 823−835

Post DL (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703-718

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Romanuk TN, Hayward A, Hutchings JA (2011) Trophic level scales positively with body size in fishes. Global Ecol Biogeogr 20: 231-240

Rubenstein DR, Hobson K (2004) From birds to butterflies: Animal movement patterns and stable isotopes. Trends Ecol Evol 19: 256-263

Semmens BX, Ward EJ, Moore JW, Darimont CT (2009) Quantifying inter-and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. PLoS ONE 4(7): e6187

Stock BC. Semmens BX (2015). MixSIAR User Manual, version 3.0. https://github.com/brianstock/MixSIAR/

Towner AV, Underhill LG, Jewell OJD, Smale MJ (2013) Environmental influences on the abundance and sexual composition of white sharks *Carcharodon carcharias* in Gansbaai, South Africa. PLoS ONE 8(8): e71197

Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB et al. (2010) Regional management units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. PLoS ONE 5(12): e15465

Wallace BP, DiMatteo AD, Bolten AB, Chaloupka MY, Hutchinson BJ et al. (2011) Global conservation priorities for marine turtles. PLoS ONE 6(9): e24510

Zuffa M, Van Grevelynghe G, De Maddalena A, Storai T (2002) Records of the white shark, *Carcharodon carcharias* (Linnaeus 1758), from the western Indian Ocean. S Afr J Sci 98: 347-349

Table 5.1 Variance component analysis from linear mixed-model analysis for white shark (*Carcharodon carcharias*) vertebral $\delta^{13}C$ and $\delta^{15}N$. Region is the highest median prey contribution for the group: KZN-KwaZulu-Natal prey, Pelagic- pelagic prey species, Cape 1-73% median contribution of Cape prey, Cape 2- 51% median contribution of Cape prey. The between-individual component (BIC) represents the total intercept variance and the within-individual component (WIC) represents the residual variance. Total niche width (TNW) is the sum of the intercept and residual variances for $\delta^{13}C$ and $\delta^{15}N$. Total BIC and total WIC are calculated by combining the intercept variances for $\delta^{13}C$ and $\delta^{15}N$ and then dividing by TNW. Proportion of WIC and BIC that explained TNW is in parentheses.

	$\delta^{13}C$				$\delta^{15}N$				Total			
Region	BIC	WIC	TNW	WIC/TNW	BIC	WIC	TNW	WIC/TNW	BIC (%)	WIC (%)	TNW	WIC/TNW
All	0.26	0.25	0.52	0.48	0.48	0.89	1.37	0.65	0.75 (40)	1.14 (60)	1.89	0.60
KZN	0.23	0.25	0.48	0.51	0.00	0.81	0.81	1.0	0.23 (18)	1.07 (82)	1.33	0.82
Pelagic	0.06	0.38	0.44	0.87	0.31	0.69	01.00	0.69	0.37 (25)	1.07 (75)	1.44	0.75
Cape 1	0.03	0.19	0.22	0.88	0.18	1.17	1.35	0.87	0.21 (13)	1.36 (87)	1.57	0.87
Cape 2	0.07	0.18	0.25	0.72	0.13	0.79	0.91	0.86	0.20 (17)	0.96 (83)	1.16	0.83

Figure 5.1 Image of a vertebral section from a 279 cm fork length female white shark (*Carcharodon carcharias*) after sample extraction along the corpus calcareum for stable isotope analysis.

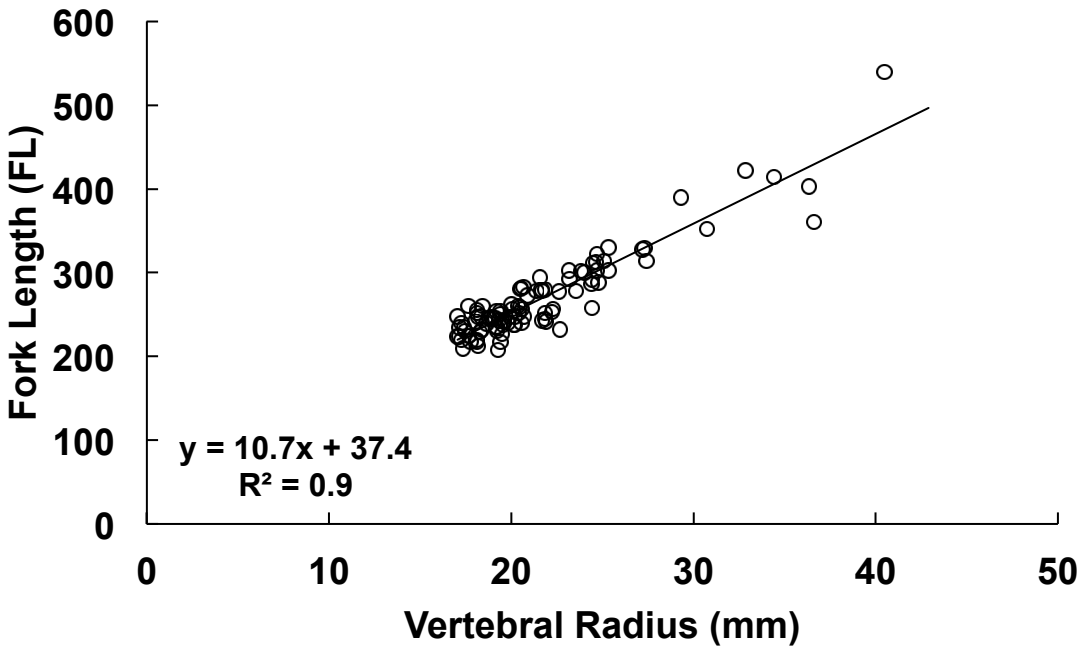

Figure 5.2 Relationship between vertebral radius and fork length for white sharks (*Carcharodon carcharias*) in the southwest Indian Ocean (n=100).

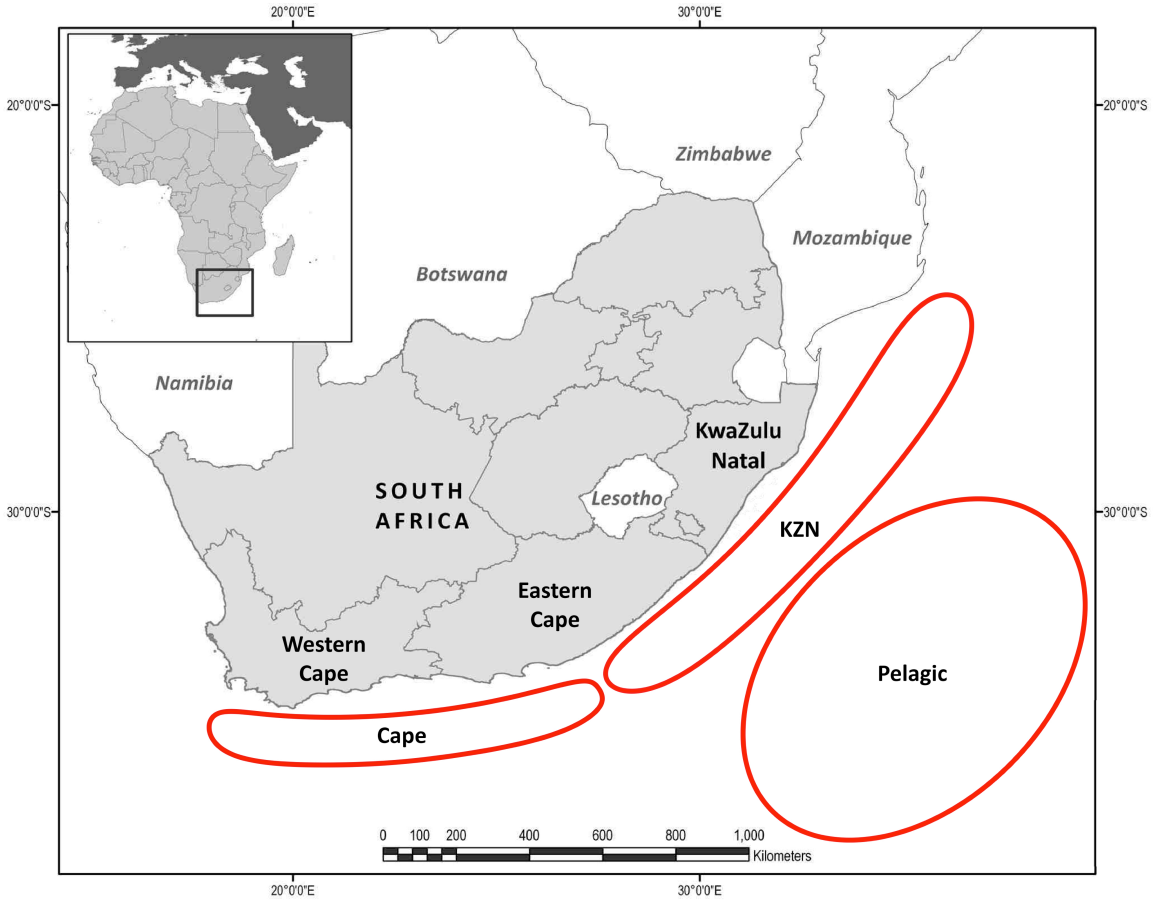

Figure 5.3 Defined ecoregions for potential prey species of white sharks (*Carcharodon carcharias*) off southern Africa used in the mixing model.

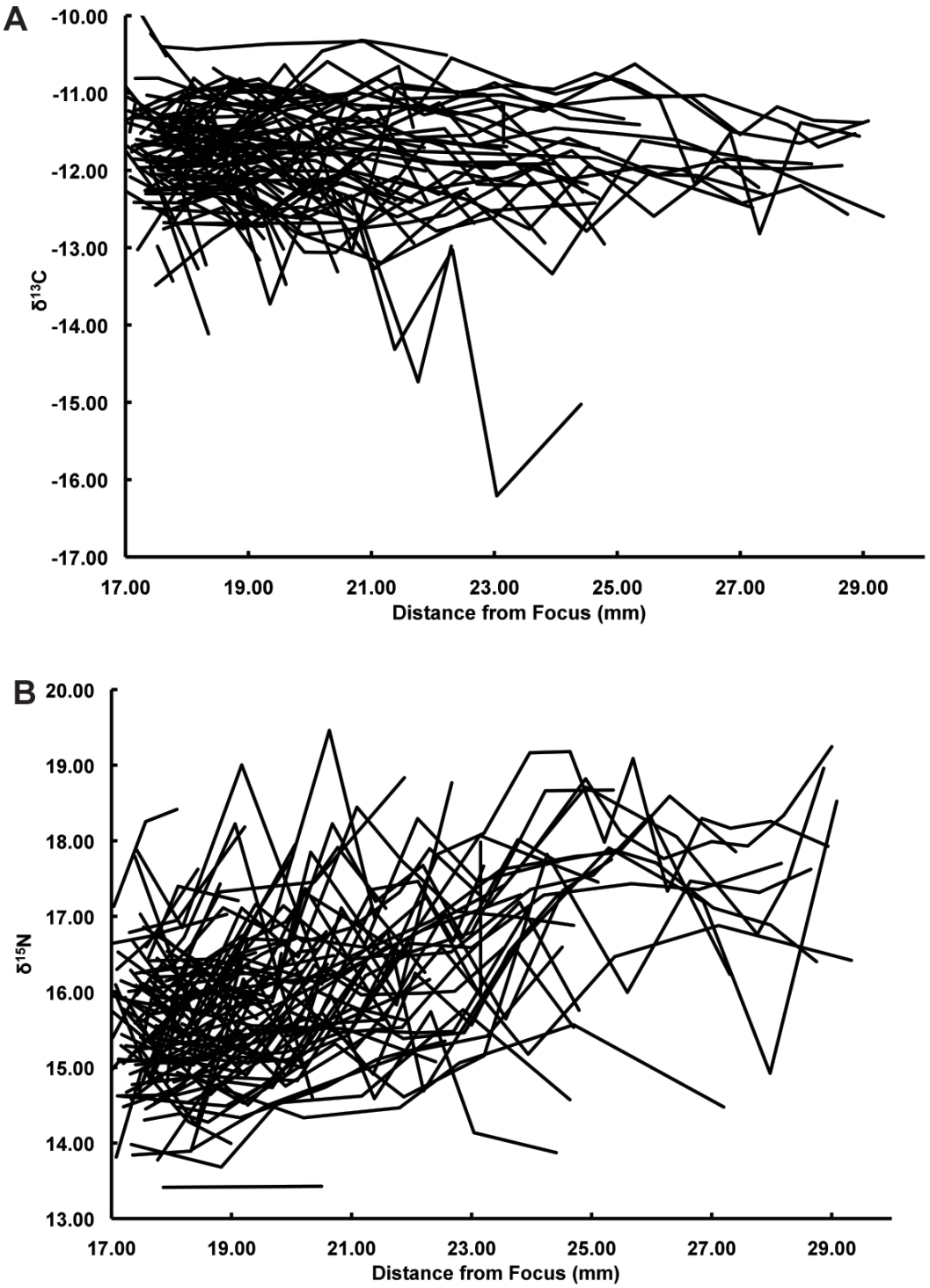

Figure 5.4 Stable isotope profiles of 100 white sharks (*Carcharodon carcharias*) from southern Africa. A single line represents each individual sampled approximately every 1–2 mm. Distance is measured from the center of the vertebra (focus). **A**- Ontogenetic $\delta^{13}C$ values for each individual, **B**- Ontogenetic $\delta^{15}N$ values for each individual.

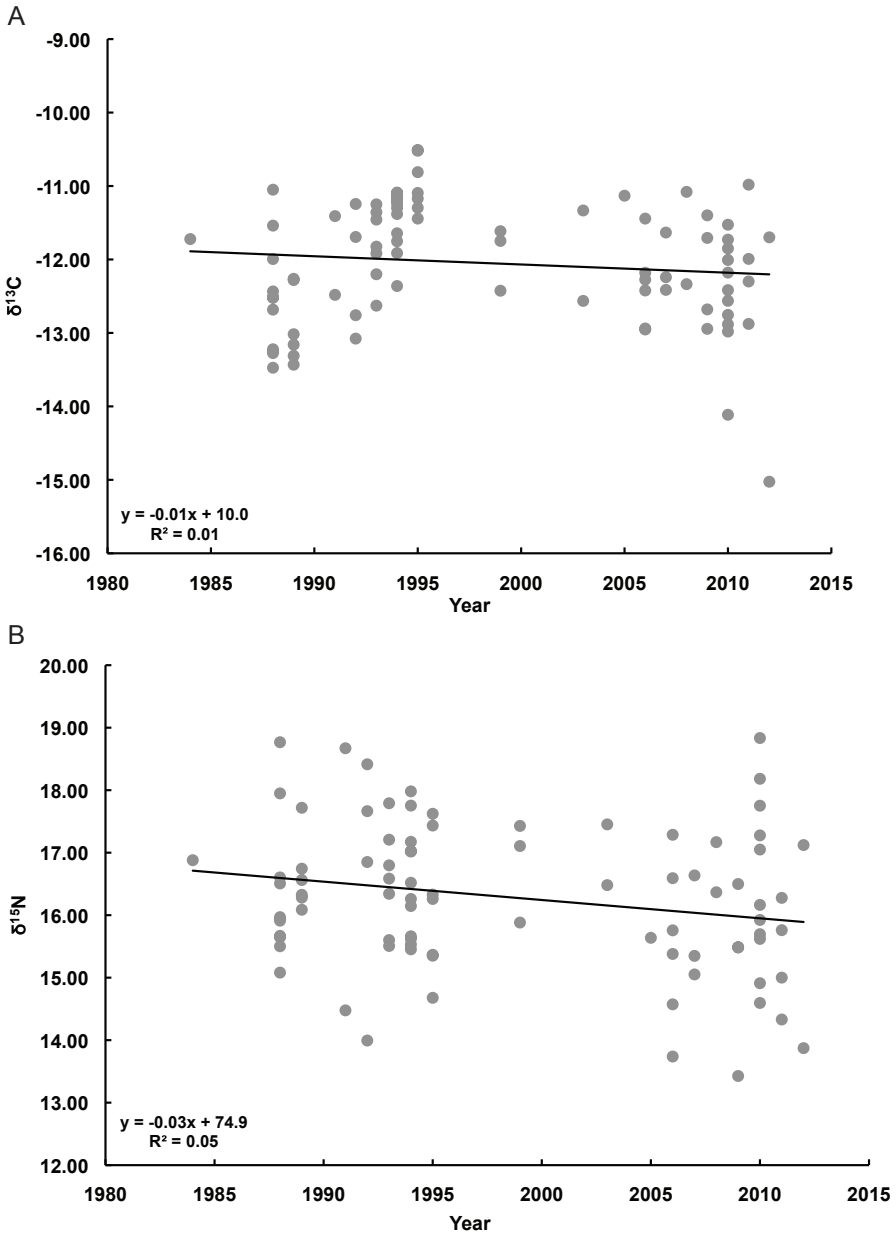

Figure 5.5 Terminal stable isotope value (**A**- $\delta^{13}C$, **B**- $\delta^{15}N$) and year of capture for 88 white sharks (*Carcharodon carcharias*) from southern Africa. Grey circles indicate individual sharks. The linear regression is represented by the black line.

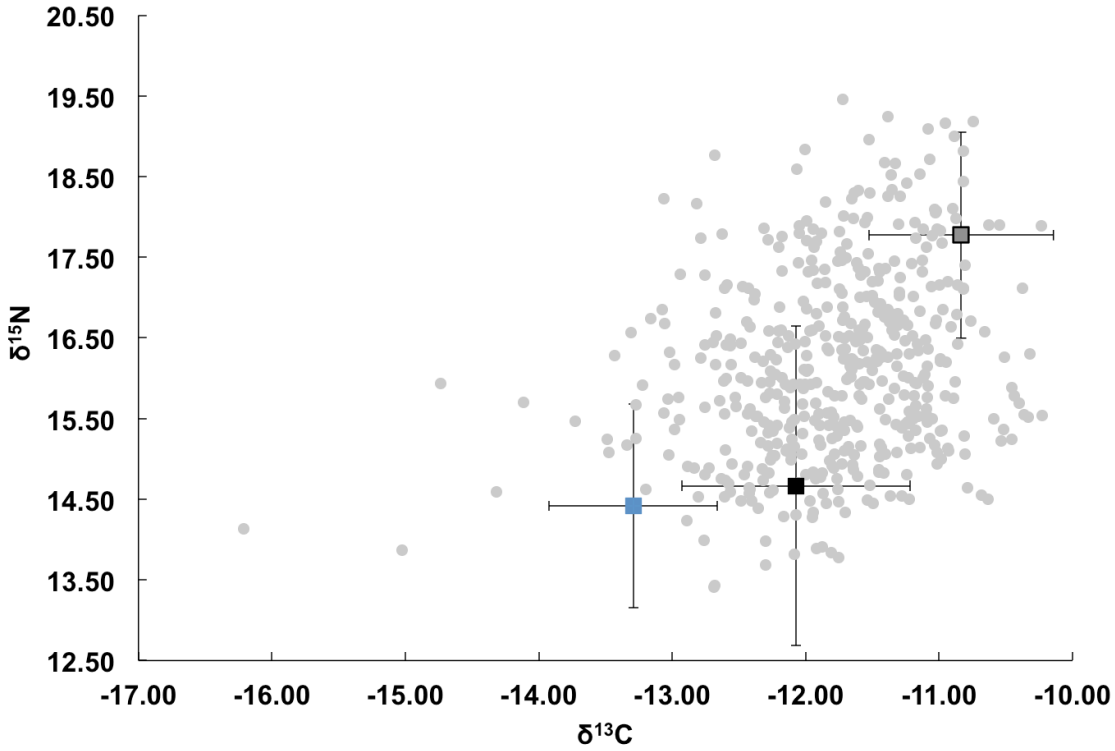

Figure 5.6 Stable isotope values for all individual points sampled from the vertebral centra of white sharks (*Carcharodon carcharias*) from southern Africa are indicated by grey circles. Regional mean (± SD) prey values adjusted to account for diet-tissue discrimination factors are represented by square symbols: black represents the KwaZulu-Natal region, blue represents the Pelagic region, and grey represents the Cape region.

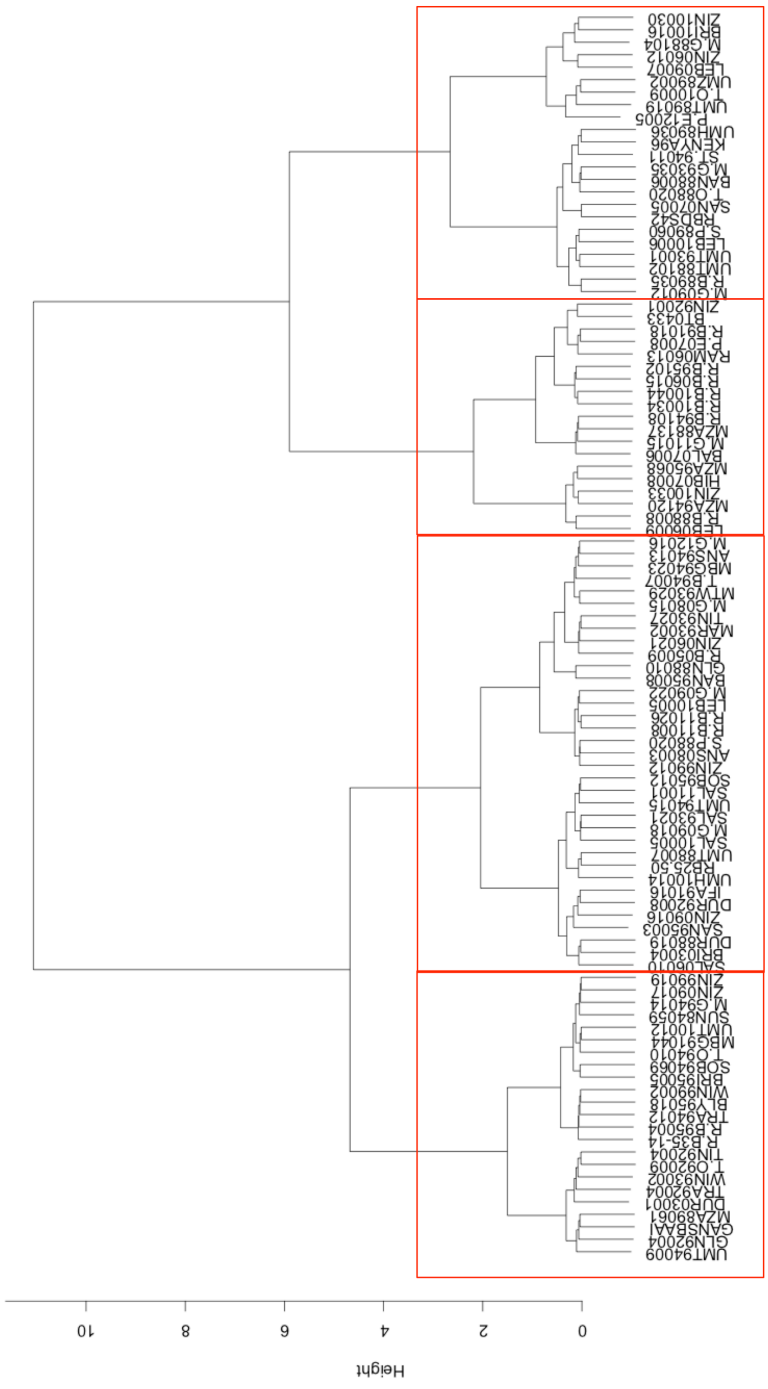

Figure 5.7 Hierarchical cluster analysis based on median prey contribution to the diet of white sharks (*Carcharodon carcharias*) as determined by the stable isotope-mixing model (MixSIAR). Red boxes indicate division of four groups.

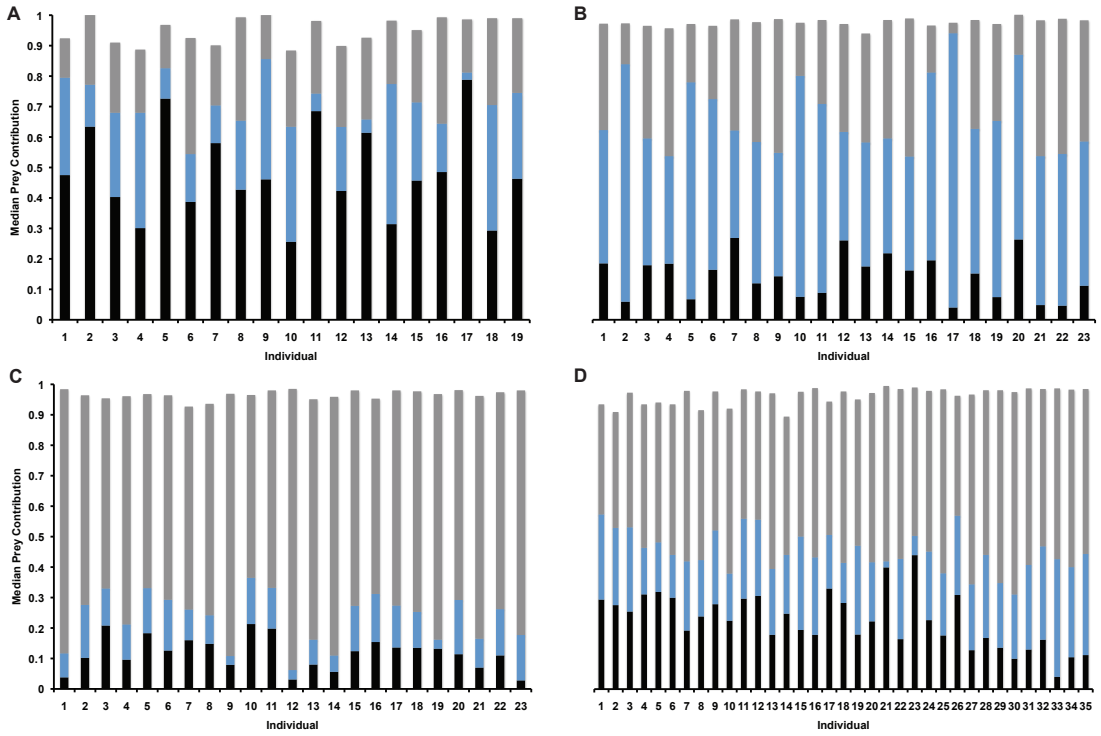

Figure 5.8 Stable isotope mixing model (MixSIAR) results with predicted median prey contribution to the diet of white sharks (*Carcharodon carcharias*) in the southwest Indian Ocean. Source prey groups were grouped according to bioregion; black representing prey from KwaZulu-Natal, blue representing Pelagic prey, and grey representing prey from the Cape region. Sharks were clustered into four groups based on hierarchical cluster analysis, **A**- Individuals with a dominant KwaZulu-Natal contribution, **B**- Individuals with a dominant Pelagic contribution, **C**- Individuals with a dominant Cape contribution (mean- 73%) **D**- Individuals with a dominant Cape contribution (mean-51%)

Table S5.1 Prey sources used to estimate mean regional $\delta^{13}C$ and $\delta^{15}N$ used in MixSIAR to assess proportional contribution of different regions to white shark vertebral tissue. Stable isotope data was obtained from the listed source and prey species were sorted into bioregion based on their distribution in the listed references.

Species	Common Name	N	$\delta^{13}C$	$\delta^{15}N$	Source	Reference
Cape Region						
Arctocephalus pusillus	Cape fur seal	139	-14.7	16.6	1	7
Boopsoidea inornata	Fransmadam	3	-15.1	15.6	2	7
Callorhinchus capensis	St. Josephs	4	-14.6	14.8	2	7, 8
Carcharias taurus	Raggie shark	3	-14.6	15.0	3	9, 10
Chrysoblephus gibbiceps	Red stumpnose seabream	1	-14.1	17.2	2	7
Chrysoblephus laticeps	Red roman	4	-14.6	15.9	2	11
Galeorhinus galeus	Soupfin	8	-14.6	15.9	2	7, 12
Pachymetopon blochii	Hottentot seabream	3	-14.7	14.9	2	13, 14
Poroderma africanum	Pajama shark	8	-12.8	16.0	2	7
Poroderma pantherinum	Leopard catshark	2	-13.0	15.6	2	7, 15
Pterogymnus laniarius	Panga seabream	4	-14.4	14.7	2	7, 16
Pelagic Region						
Acanthocybium solandri	Wahoo	8	-17.0	12.8	4	2, 17
Coryphaena hippurus	Dorado (common dolphinfish)	4	-17.4	12.0	4	2, 18
Cubiceps whiteleggii	Shadow driftfish	3	-17.5	11.7	1	19
Decapterus russelli	Indian scad	1	-16.8	10.8	1	20
Diaphus knappi	Lanternfish	4	-17.9	12.3	1	21
Euthynnus affinis	kawakawa	1	-16.6	13.1	4	7, 22
Globicephala macrorhynchus	short-finned pilot whale	4	-16.7	12.7	1	23
Istiophorus platypterus	Indo-Pacific sailfish	1	-18.6	11.7	4	7, 24
Isurus oxyrinchus	Shortfin mako	29	-16.7	14.6	5	10
Lepidopus caudatus	Frostfish	3	-18.5	11.0	1	16

Polyipnus indicus	Hatchetfish	3	-18.3	12.5	1	16
Polymetme corythaeola	Lightfish	3	-17.7	13.3	1	16
Pomatomus saltatrix	Bluefish	5	-16.1	13.7	1	7
Prionace glauca	Blue shark	31	-17.5	13.6	5	7, 25
Pteroplatytrygon violacea	Pelagic stingray	1	-14.5	14.6	2	56
Rhincodon typus	Whale shark	3	-17.3	9.9	3	10
Sardinops sagax	South African sardine	6	-16.7	10.8	3	27
Scomber japonicus	Chub mackerel	5	-16.5	11.4	1	7, 17
Scomberomorus commerson	Couta	14	-16.9	12.6	4	7, 17
Scomberomorus plurilineatus	Natal Snoek	1	-16.9	13.0	1	7, 17
Stenella attenuata	Spotted dolphin	1	-16.2	12.0	1	23
Synagrops japonicus	Japanese (Blackmouth) splitfin	3	-17.9	10.9	1	21
Thryssa vitrirostris	Orangemouth anchovy	5	-16.4	12.7	1	28
Thunnus albacares	Yellowfin tuna	1	-17.6	12.2	1	7, 29
Trachurus delagoa	African scad	2	-17.2	11.5	1	16
Trichiurus lepturus	Largehead hairtail	5	-16.5	12.6	1	16
Tursiops truncatus	Bottlenose dolphin	3	-15.8	12.9	1	13
KwaZulu-Natal Region						
Aprion virescens	Kakaap	6	-17.2	11.8	4	7, 29
Argyrosomus thorpei	Squaretail kob	3	-16.1	12.5	1	7, 29
Atrobucca nibe	Blackmouth croaker	5	-16.6	12.1	1	30
Balistapus undulatus	Orange lined trigger fish	1	-17.7	9.9	4	16
Carangoides fulvoguttatus	Fulvi	6	-17.4	12.3	4	7, 21
Caranx ignobilis	Iggie (giant trevally)	3	-17.2	13.2	4	7, 30
Caranx sexfasciatus	Bigeye kingfish	4	-17.0	12.2	4	7, 21
Carcharhinus leucas	Bull shark	8	-15.2	11.9	3	7, 31, 32
Carcharhinus obscurus	Dusky shark	64	-15.6	13.7	3	7

Carcharhinus plumbeus	Sandbar shark	6	-15.2	15.0	3	31,33
Carcharias taurus	Raggie shark	26	-14.3	15.7	3	9
Cephalopholis miniata	Coral grouper	1	-16.9	11.7	4	21
Cephaloscyllium sufflans	Swell shark	3	-16.0	12.6	3	21
Chrysoblephus puniceus	Slinger seabream	5	-17.5	12.0	1	7, 34
Cruriraja triangularis	Roughnose legskate	4	-16.9	12.4	1	35
Cynoglossus attenuatus	Fourline tonguesole	5	-15.9	11.3	1	7
Cynoglossus lida	Rough scale tongue fish	7	-17.0	13.5	1	21
Dalatias licha	Kitefin shark	3	-16.2	13.8	1	31
Galeocerdo cuvier	Tiger shark	18	-16.1	13.0	3	7, 31
Halaelurus lineatus	Banded catshark	5	-16.2	12.0	3	10
Himantura gerrardi	Sharpnose ray	3	-15.0	12.7	1	7, 34
Himantura leoparda	Honeycomb ray	2	-15.0	12.3	1	7, 34
Johnius dorsalis	Small kob	5	-15.9	12.4	1	7, 36
Johnius fuscolineatus	Bellfish	5	-15.9	12.7	1	36
Leiognatus equulus	Common ponyfish	5	-17.1	12.4	1	21, 34
Lutjanus gibbus	Humpback snapper	3	-14.8	11.5	1	30
Pellona ditchella	Shad	2	-16.3	11.8	1	37
Rhabdosargus sarba	Natal stumpnose	1	-15.6	9.9	4	7, 34
Rhinobatus leucospilus	Greyspotted guitarfish	6	-17.2	10.0	1	35
Rhizoprionodon acutus	Milk shark	5	-15.8	14.5	3	7, 31
Rhynchobatus djiddensis	Giant guitarfish	3	-15.6	12.1	3	7, 34
Secutor insidiator	Pugnose ponyfish	5	-16.5	11.9	3	30
Seriola dumerili	Amberjack	3	-17.4	11.9	4	7
Sphyraena acutipinnis	Sharpfin barracuda	4	-16.1	12.0	1	21
Sphyraena jello	Pickhandle	4	-16.5	14.0	4	7, 21
Sphyrna lewini	Scalloped hammerhead	38	-15.6	14.8	3	7, 34
Torpedo sinuspersici	Marbled electric ray	11	-14.8	12.7	1	30

References

1. Hussey NE, unpublished data
2. De Nacker L, unpublished data
3. Hussey NE, MacNeil MA, Siple MC, Popp BN, Dudley SFJ et al. (2015) Expanded trophic complexity among large sharks. Food Webs 4: 1-7
4. Daly R, Froneman PW, Smale MJ (2013) Comparative feeding ecology of bull sharks (*Carcharhinus leucas*) in the coastal waters of the southwest Indian Ocean inferred from stable isotope analysis. PLoS ONE 8(10): e78229
5. Kiszka JJ, Aubail A, Hussey NE, Heithaus MR, Caurant F et al. (2015) Plasticity of trophic interactions among sharks from the oceanic south-western Indian Ocean revealed by stable isotope and mercury analyses. Deep-Sea Res Pt 1 96: 49-58
6. Oosthuizen WH (1991) General movements of South African (Cape) fur seals *Arctocephalus pusillus pusillus* from analysis of recoveries of tagged animals. S Afr J Mar Sci 11: 21-29
7. Mann BQ (Ed) (2013) Southern African marine linefish species profiles. Oceanographic Research Institute, Durban, South Africa 357 p.
8. Freer DWL, Griffiths CL (1993) Estimation of age and growth in the St. Joseph *Callorhinchus capensis* (Dumeril). S Afr J Mar Sci 13: 75-82
9. Dicken M, Smale M, Booth A (2006) Spatial and seasonal distribution patterns of the ragged-tooth shark *Carcharias taurus* along the coast of South Africa. Afr J Mar Sci 28: 603-616
10. Compagno LJV (2001) Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Volume 2. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Catalogue for Fishery Purposes. No. 1, Vol. 2. Rome, FAO. 269 p.
11. Kerwath SE, Götz A, Attwood CG et al. (2007) Movement pattern and home range of Roman *Chrysoblephus laticeps*. Afr J Mar Sci 29: 93-103
12. Walker, TI (1999) *Galeorhinus galeus* fisheries of the world. In: Shotton R (ed) Case Studies of Management of Elasmobranch Fisheries, FAO fisheries technical paper no 378/2 pp728-772
13. Pulfrich A., Griffiths CL (1988) Growth, sexual maturity and reproduction in the hottentot *Pachymetopon blochii* (Val.). S Afr J Mar Sci 7: 25-36
14. van der Elst (1981) A Guide to the Common Sea Fishes of Southern Africa. Cape Town; Struik: 367 pp.
15 Heemstra P, Heemstra E (2004) Coastal Fishes of Southern Africa. Grahamstown, South Africa: NISC, SAIAB. 488 p.
16. Smith MM, Heemstra PC (eds), *(*2003*)* Smiths' Sea Fishes. *Struik Publishers,* Johannesburg.
17. Collette BB, Nauen CE (1983) FAO Species Catalogue Vol. 2 Scombrids of the world an annotated and illustrated catalogue of Tunas, Mackerels, Bonitos and related species know to date. FAO Fish Synopsis 2: 137
18. Beardsley GL Jr (1967) Age, growth, and reproduction of the dolphin, *Coryphaena hippurus*, in the Straits of Florida. Copeia 2: 441-451
19. Froese R, Pauly D (2015) FishBase. www.fishbase.org, accessed January 16, 2016
20. Food and Agriculture Organization of the United Nations-Fisheries Global Information System (2001) Species fact sheets: *Decapterus russelli*. 30/05/2005
21. Fischer, W. and G. Bianchi (eds) (1984) FAO species identification sheets for fishery purposes. Western Indian Ocean; (Fishing Area 51). Danish International Development Agency (DANIDA). Rome, Food and Agricultural Organization of the United Nations, vols 1-6
22. Griffiths SP, Kuhnert PM, Fry GF, Manson FJ (2009) Temporal and size-related variation in the diet, consumption rate, and daily ration of mackerel tuna (*Euthynnus affinis*) in neritic waters of eastern Australia. ICES J Mar Sci, 66: 720-733
23. Findlay KP, Best PB, Ross GJB, Cockcroft VG (1992) The distribution of small odontocete cetaceans off the coasts of South Africa and Namibia. S Afr J Mar Sci 12: 237-270
24. Nakamura I (1983) Systematics of the billfishes (Xiphiidae and Istiophoridae). Publ Seto Mar Biol Lab 28: 255-396
25. Nakano H, Stevens JD (2008) The biology and ecology of the blue shark, *Prionace glauca*. In: Camhi MD, Pikitch EK, Babcock EA (eds) Sharks of the Open Ocean: Biology, Fisheries and Conservation. Blackwell Publishing, Oxford, pp 140-151

26. Mollet HF, Ezcurra JM, O'Sullivan JB (2002) Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Mar Freshw Res 53: 531-541
27. Armstrong MJ, Chapman P, Dudley SFJ, Hampton I, Malan PE (1991) Occurrence and population structure of pilchard *Sardinops ocellatus*, round herring *Etrumeus whiteheadi* and anchovy *Engraulis capensis* off the east coast of southern Africa. S Afr J Mar Sci 11: 227-249
28. Mualeque D, Santos J (2011) Biology, fisheries and distribution of *Thryssa vitrirostris* (Gilchrist & Thompson 1908) and other Engraulidae along the coast of the Sofala Bank, western Indian Ocean. Afr J Mar Sci 33: 127-137
29. Smale MJ (1986) The feeding habits of six pelagic and predatory teleosts in eastern Cape coastal waters (South Africa). J Zool 1: 357-409
30. van der Elst R (1993) A guide to the common sea fishes of southern Africa. (3rd Ed.). Struik Publishers, Cape Town. 398 p.
31. Compagno LJV (1984) Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 2. Carcharhiniformes. FAO species catalogue. Rome, FAO Vol 4, Pt. 2: 251-655
32. Cliff G, Dudley SFJ (1991) Sharks caught in the protective gill nets off Natal, South Africa. 4. The bull shark *Carcharhinus leucas* (Valenciennes). S Afr J Mar Sci 10: 253-270
33. Cliff G, Dudley SFJ, Davis B (1988) Sharks caught in the protective gill nets off Natal, South Africa. 1. The sandbar shark *Carcharhinus plumbeus* (Nardo). S Afr J Marine Science 7: 255-265
34. Mann B, Celliers L, Fennessy S, Bailey S, Wood AD (2006) Towards the declaration of a large marine protected area: a subtidal ichthyofaunal survey of the Pondoland coast in the Eastern Cape, South Africa. S Afr J Mar Sci 28: 535-551
35. Compagno LJV, Ebert DA, Smale MJ (1989) Guide to the sharks and rays of southern Africa. New Holland (Publ.) Ltd., London. 158 p.
36. Sasaki K (1997) Resurrection of two East African species of *Johnius* (Perciformes, Sciaenidae). Ichthyol Res 44: 311-315
37. Whitehead PJ (1985) FAO Species Catalogue. Vol. 7. Clupeoid fishes of the world (suborder Clupeioidei). An annotated and illustrated catalogue of the herrings, sardines, pilchards, sprats, shads, anchovies and wolf-herrings. FAO Fish. Synop. Rome: FAO 125(7/1): 1-303

CHAPTER 6: THE LAST FRONTIER: CATCH RECORDS OF WHITE SHARKS (*CARCHARODON CARCHARIAS*) IN THE NORTHWEST PACIFIC OCEAN[4]

INTRODUCTION

Details on the population status and spatial/temporal distribution of threatened species are critical to focus conservation efforts (Cooke 2008, Simpfendorfer et al. 2011). Typically, species that have globally distributed populations are exposed to a range of region-specific threats and pressures and consequently management actions require data for each region (Halpern et al. 2007, Wallace et al. 2010, Wallace et al. 2011). Regional declines of large marine predators including elasmobranchs have been documented (Dulvy et al. 2008, 2014), with potential cascading effects on marine food webs (Ferretti et al. 2010). For many large elasmobranch species, data on spatial and temporal distributions are limited due to their migratory behavior, the relative rarity of sightings, and the nature of the environment they inhabit (Wilson et al. 2006, MacNeil et al. 2012).

The white shark (*Carcharodon carcharias*) is a large (maximum size 6 m, (Mollet et al. 1996, Castro 2012)) marine apex predator with a global distribution, occurring in temperate, sub-tropical, and tropical waters (Bonfil et al. 2005, Weng et al. 2007a, Domeier 2012a, Duffy et al. 2012). The life history characteristics of white sharks (e.g., natural low abundance, slow growth, late maturity, low fecundity) make them vulnerable to exploitation (Francis 1996, Smith et al. 1998). Population estimates conducted in California (Dewar et al. 2013), South Africa (Cliff et al. 1996, Towner et al. 2013a), and Australia (Strong et al. 1996) indicate relatively low regional population sizes with genetic diversity constricted by philopatric behavior (Blower et al. 2012). White sharks

[4] Christiansen HM, Lin V, Tanaka S, Velikanov A, Mollet HF, Wintner SP, Fordham SV, Fisk AT, Hussey NE (2014) The last frontier: catch records of white sharks (*Carcharodon carcharias*) in the northwest Pacific Ocean. PLoS ONE 9(4): e94407

are classified as threatened (globally Vulnerable) by the International Union for Conservation of Nature (IUCN), listed on the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) Appendix II, listed on both Appendix I and II of the Convention on Migratory Species (CMS), and are protected by national legislation in Australia, Canada (Atlantic Ocean), Croatia, European Union, Maldives, Malta, Mexico, Namibia, New Zealand, South Africa, and in all United States waters (except in the Western Pacific) (Camhi et al. 2009, Kyne et al. 2012). For the countries of the Northwest Pacific Ocean, participation in CITES and CMS is variable, while none appear to impose complete national white shark protection. Specifically, the only domestic, white shark-specific conservation measure identified in the region applies in China, but permits with unclear conditions may allow for some take (Table 6.1).

The white shark is a charismatic species and consequently one of the most studied and protected shark species (Domeier 2012b); however, there is still much to learn about their basic biology and ecology. The majority of recent research on white shark movement and feeding ecology has focused on regional population hotspots or aggregation sites in the Northeast Pacific, South Africa, and surrounding Australia (including New Zealand and New Caledonia), with only a few studies examining the lesser-known populations of the Mediterranean (Fergusson 1996, Gubili et al. 2010, De Maddalena and Heim 2012) and the Northwest Atlantic (Skomal et al. 2012) (Figure 6.1). For the Northwest Pacific region, there is a paucity of available data on white sharks with only a few reports of incidental captures and white shark-human interactions (bites) from Japan (Nakano and Nakaya 1987, Uchida et al. 1987, Nakaya 1994, Nakaya 1996, Uchida et al. 1996, Tanaka et al. 2011) and Russia (Velikanov 2010, Dolganov 2012).

This disparity in research focus is so large that the Proceedings book of the last international white shark symposium (International White Shark Symposium 2010) contained no data for the Northwest Pacific Ocean (Domeier 2012b). Currently, the frequency of occurrence and geographical extent of the population of white sharks in this region is unknown. Based on repeated long distance migrations recorded in South Africa and the Northeast Pacific (Bonfil et al. 2005, Bonfil et al. 2010, Duffy et al. 2012, Domeier and Nasby-Lucas 2012), it is possible that white sharks transit to the Northwest Pacific from other regional populations, such as Australia, however, evidence suggests that white sharks caught off Japan form a genetically distinct population that has a vastly different growth rate from the Northeast Pacific and South African populations (Tanaka et al. 2011). It is therefore likely that there is a separate resident population of white sharks inhabiting the Northwest Pacific region.

Considering the conservation concern for white sharks, the lack of protective measures in the Northwest Pacific region and the limited data available for the associated population, the aims of this study were to; i) provide a comprehensive record of the distribution of white shark catches and sightings in the Northwest Pacific Ocean, ii) describe spatio-temporal patterns in white shark occurrence, iii) examine relative population trends over the past 60 years, iv) document size and sex based trends, and v) provide details on the reproductive biology of females. These data provide a baseline to stimulate further research and to inform regional management for this little known population.

METHODS

The study region of the Northwest Pacific Ocean was defined as the waters surrounding Russia, the Republic of Korea, Japan, China, Taiwan, the Philippines and Vietnam. A literature search was conducted using the ISI Web of Science. Search terms included: "white shark", "*Carcharodon carcharias"* and "Russia or Republic of Korea or Japan or China or Taiwan or Philippines or Vietnam". This resulted in 12 studies, six of which contained no relevant data for the Northwest Pacific Ocean and were therefore excluded. Regional observations of white sharks were defined as any records of fishery landings/discards, interactions with humans (bites), or museum specimens. To document white shark observations from media reports and websites, a search was conducted using Google. Personal communications and observations by the authors were also included. In Japan, the Ibaraki Prefectural Oarai Aquarium works directly with local fishermen, including those involved in set net fisheries, to document white shark catches.

Data Analysis

Data were filtered to remove duplicate observations. The amount of information recorded for each white shark observation ranged from basic data that included only the country of capture to detailed records that included date of capture, city landed, method of capture, size (cm total length - TL), weight (kg), sex, and if the individual was pregnant. In most instances, the locations of white sharks were based on landing sites rather than actual catch locations (see Table S6.1). To examine population trends over time, a generalized linear model was fit to the observation data period to examine the magnitude of change in population relative to a set of reference dates following the approach developed by McPherson and Myers (2009). Values equal to one or less suggest

a stable or increasing population, while values larger than one are indicative of a decline in relative abundance. This model enables testing of sensitivity to changes in observer effort over the reference period of 1951-2011. Consequently, +75%, 0% and -75% changes in observer effort were selected to incorporate a wide range of potential observer variation.

We documented whether length and weight were measured by scientifically trained staff or estimated by laypeople/untrained fishermen, from photos, or by jaw size. When both an estimate from a fishermen and calculated TL from jaws or photos were available, the TL from the latter was used. When multiple weight estimates were available the lowest estimate was retained. The length-weight relationship was calculated using the equation $\log W = \log a + b^{*}\log TL$ where W is weight (kg), TL is total length (cm) and a and b are constants (where $\log a$ is the intercept and b is the slope). Significant differences by sex were tested using an analysis of covariance (ANCOVA) on log-transformed data. Since estimated weights were used for several individuals (Table S6.1), the validity of the data was tested using an ANCOVA with length-weight data obtained from white sharks caught in beach protection nets off KwaZulu-Natal, South Africa (KwaZulu-Natal Sharks Board, unpublished data), as well as global length-weight data collected by trained scientists (Fergusson 1996, Francis 1996, Ezcurra et al. 2012, see references therein). Pregnant females were excluded from this analysis.

Due to the number of observations where sex data were available (113, 47%), the sex ratio for each country was determined, but further statistical analysis, e.g. segregation by size and sex or temporal differences by sex, were not conducted. Embryos of pregnant individuals were categorized by size as early term (< 40 cm TL), mid-term (between 40

and 100 cm TL) or full term (> 100 cm TL) (Francis 1996, Uchida et al. 1996, Mollet et al. 2000). For individuals where embryo size was described by stage, this description was maintained. To estimate gestation length, data from embryos and free-swimming juveniles from the Northern and Southern Hemispheres (Table S6.2) were combined on one time scale, where January equals month 0 in the Northern Hemisphere and July equals month 0 in the Southern Hemisphere. Linear regression was performed on the embryo size versus month of capture data and gestation length was determined using the slope of the regression, and the size at birth (we used 126 cm TL, the smallest free-swimming individual in the Northwest Pacific).

RESULTS

A total of 248 white shark observations were documented; after the removal of duplicates 240 reliable observations remained (Table S6.1). Records of white sharks were found in seven countries ranging from the most northerly in temperate waters off Russia to the most southerly in tropical waters off Vietnam (Figure 6.2A, 6.3), spanning a straight line latitudinal distance of 4,300 km. Location of landing was recorded for 169 observations, while the remaining reports only included the country of landing (Figure 6.2A, B). Observations of white sharks occurred between 1951 and 2012; the year of capture was recorded for 212 animals. The number of observations increased over time, with two peaks occurring in 1992 and 2009 and no observations documented between 1960 and 1974 (Figure 6.4A). Correct species identification for the 240 white sharks was confirmed through records from trained scientists, personal observations, preserved remains, photographs or media reports (Figure 6.5; Table S6.1). The method of capture was documented for 79 observations with 56 animals caught in set nets, 12 by set lines, 3

each by either gillnet, seine or trawls and one each by harpoon and crab basket (Table S6.1). The majority of these captures occurred in Japan (76) with 25 occurring in the set net fishery off Ibaraki and 11 in the set line fishery surrounding Okinawa. Of these captures, most sharks were caught in spring (April-June) (38), followed by winter (January-March) (18), summer (July-September) (12), and autumn (October-December) (10).

When considering all regional observations, month of capture was reported for 146 animals (Figure 6.6). White sharks were reported across all months and seasonal trends mirrored those of documented fishery captures in Japan with spring having the highest number of observations (59), followed by winter (39), summer (24), and autumn (24). In the more northern countries (Russia and the Republic of Korea) white sharks were not observed in autumn and early winter from October-January (Figure 6.6A). For Japan, white sharks were observed across all months of the year with a peak between April-May (Figure 6.6B). In the most southerly countries (China, Taiwan, the Philippines, and Vietnam) white sharks were observed in all months except during summer (July-August) (Figure 6.6C).

There were two time periods where focused white shark monitoring occurred in Japan (1990-1994 and 2005-2009). To avoid any biases caused by increased observation effort these data points were removed prior to population trend analysis. When accounting for no change in observer effort over time, the population trend estimate indicated that the relative abundance of white sharks has been stable or increasing in the region until recently (2007) (Figure 6.4B). Parameterization of a 75% decrease in observer effort indicated the relative abundance of white sharks has remained stable or

increased throughout the reference period, while a 75% increase in observer effort indicated a minor decline in relative abundance since 2002.

Size of animal (TL) was recorded for 175 observations (Figure 6.2C; Table S6.1). For this study, sharks whose weight and total length were estimated are identified in Table S6.1. The smallest recorded shark measured 126 cm TL and was caught in Primorye, Russia, while the largest shark was landed in Seven Star Lake, Taiwan and was estimated to be 670-700 cm TL (Figure 6.2C). The largest accurately measured shark was caught in the East China Sea and measured 602 cm TL. White sharks <200 cm TL were observed in Russia (n=1)(September), the Republic of Korea (1), Japan (2) (July) and Vietnam (1) (June). Weight was measured or estimated for 162 white shark observations (Table S6.1). The lowest weight recorded was 16 kg (for the 126 cm TL specimen-Primorye, Russia), while the largest estimated weight was 3000 kg for a 520 cm TL shark caught in Hikari City, Japan. The most reliable weight for the heaviest animal documented was 2530 kg, a 555 cm TL shark that was recorded by a fishery wholesaler in NingPuo, China. There was no significant difference in the slope of the length-weight regression between females and males, so data were combined (interaction of length and sex: F=0.3 and P=0.59). The intercept for females was higher than males indicating that females grow larger than males. When testing between combined sex length-weight data for the Northwest Pacific and global data, no significant difference between slopes was detected (interaction of length and region: F=0.06 and P=0.81). The relationship between length and weight was expressed as: Northwest Pacific $W=1.04e^{-5}TL^{3.008}$ (r^2=0.72), global data $W=5.86e^{-7}TL^{3.476}$ (r^2=0.84). A regression for all data was calculated as $W=1.61e^{-6}TL^{3.309}$ (r^2=0.85) (Figure 6.7).

Sex was recorded on 113 occasions. A total of 53 males and 60 females were documented (Figure 6.8; Table S6.1) of which 11 (18.3%) were pregnant and one individual was suspected to be pregnant (Figure 6.9; Table 6.2). The observed sex ratio was not significantly different from 1:1 for all countries combined (1:1.1, X^2=0.43, p=0.51) or individually China (1:2.8, X^2=3.3, p=0.07), Japan (1:0.7, X^2=2.3, p=0.13) and Republic of Korea (1:3, X^2=1.0, p=0.32), while it was significantly biased towards females for Taiwan (1:4, X^2=7.2, p<0.01). Sex was reported for one shark in Russia (male) and for none in Vietnam and the Philippines.

Pregnant females ranged in size from 450-600 cm TL and contained an average of six embryos (ranging from egg cases to 10 embryos) (Table 6.2). Egg cases were found in females captured in early autumn, mid-term embryos were present in winter, and full-term embryos (130-150 cm TL) were present in spring (Table 6.2). The relationship between embryo size and month of capture predicted a gestation period of 20 months (n=9, slope=6.4 cm/month, SE=1.0 cm/month, p<0.01, r^2= 0.84) (Figure 6.10). Pregnant white sharks were geographically separated during the various stages of gestation; egg cases and early term embryos were present in individuals from the southern locations of Taiwan and Okinawa, whereas full term embryos were found in individuals mostly captured near mainland Japan.

DISCUSSION

Determining the spatial and temporal distribution of threatened species is a critical first step for effective conservation measures. This is the first study to collate and present observations of white sharks in the Northwest Pacific Ocean in order to define the geographic extent of the population. While we do not assume that we obtained all records

in the region over the past 60 years, these observations provide important baseline data for this little known population, providing a benchmark to instigate further research for population-specific conservation and management efforts.

The observations of white sharks in the Northwest Pacific increased over time and showed a bimodal trend that coincided with focused monitoring of catches (1990-1994; Nakaya 1994) and sampling of white sharks by Ibaraki Prefectural Oarai Aquarium staff (2005-2009; Tanaka et al. 2011) in Japan, and consequently represent a bias in observed trends. Aside from these two periods, there was no formal monitoring program of white shark catches and actual observations may be higher due to fishermen releasing individuals at sea and/or not reporting catches (Nakaya 1994). The number of observers reporting large white sharks in this region have likely increased over time due to a combination of improved fishing gear/effort and increasing media coverage due to technological advances and heightened public interest in large marine predators. However, smaller individuals likely do not elicit the same media response and catches may go unreported or may be misidentified with other species in the family Lamnidae, resulting in lower observation numbers for this size class (Weng and Honebrink 2013). Additionally, during the study period there was no requirement to report catches of white sharks, which may have affected observer effort. When accounting for an increase, no change, and decrease in observer effort over the study period within the generalized linear model, minimal effects of observer effort on model results were observed for all scenarios. Overall the population trend analysis, which identifies changes in relative abundance and not absolute population estimates, found that the Northwest Pacific population was relatively stable. Evidence for a recent declining trend in relative

abundance of sharks with 75% increase in observer effort, identifies that it is important to enact ongoing monitoring to detect if this decline continues. Previously this analysis has documented an increase in white shark abundance off the coast of Massachusetts (Skomal et al. 2012), and steep declines in relative abundance of white shark in the eastern Adriatic and eastern Canada (McPherson and Myers 2009).

There are no directed fisheries for the relatively rare white shark; however, bycatch in artisanal and commercial fisheries has been reported from the coast of California (Lowe et al. 2012), Mexico (Santana-Morales et al. 2012), Australia (Malcolm et al. 2001), and in beach protection programs off Australia (Malcolm et al. 2001) and South Africa (Cliff and Dudley 2011). In the current study, the majority of the animals categorized as bycatch were between 300-580 TL, which contrasts with data from the above regions where most animals were young-of-the-year or juveniles (<300 cm TL) (Malcolm et al. 2001, Lowe et al. 2012, Santana-Morales et al. 2012). It has been proposed that larger white sharks (> 300 cm TL) are less susceptible to entanglement in fishing gear (Cliff and Dudley 2011, Lowe et al. 2012), but this finding is not supported by our data for the Northwest Pacific. Given that in this study the largest numbers of white sharks documented as fisheries bycatch were caught in Japan, specifically the set net fishery off Ibaraki, further research including biological sampling, could shed light on this discrepancy and other related questions of regional population size and distribution.

Temperatures in this region vary both seasonally and geographically, ranging from 0°C at northern latitudes off Russia to 28°C off Vietnam (Locarnini et al. 2010, Payne et al. 2012). White sharks were not observed during autumn and early winter in the most northerly latitudes, similar to the absence of white sharks in the northern latitudes of

the Northeast Pacific (Martin 2004). Similarly, sharks were absent from the most southern latitudes during July-August, suggesting they have a preferred temperature niche. White sharks have been documented in water temperatures from 3-27°C (Martin 2004, Bonfil et al. 2005, Weng et al. 2007a, Domeier 2012a, Domeier and Nasby-Lucas 2012, Francis et al. 2012); but typically spend most of their time in a narrow range of water temperatures, from 16.4-24.7°C (Adams et al. 1994, Santana-Morales et al. 2012, Dudley 2012, Towner et al. 2013b, Weltz et al. 2013). As the SST off Japan encompasses these temperature ranges (Moriyasu 1972, Park et al. 2011), it is possible that sharks move along the Japanese coastline concurrent with the seasonal change in SST. Alternatively, white sharks may inhabit these temperatures due to prey availability (Weltz et al. 2013). While there are documented seal rookeries along the Japanese coastline (Mizuno et al. 2001, Han et al. 2010) further research is required to determine the extent white sharks utilize them as a food source. Regardless of the cause of habitat preference (temperature or prey availability) these data suggest that Japan is an important aggregation site for the regional population similar to Central California and Guadalupe Island in the Northeast Pacific (Domeier 2012a, Jorgensen et al. 2012).

Prior to this study, the largest white shark on record worldwide was reported to be 600 cm TL and was caught in Western Australia (Castro 2012). One shark in this study was estimated to be 670-700 cm TL; however, examination of the jaw dimensions revealed it to be smaller (approximately 600 cm TL; Author Observation). The longest reliably measured shark in this study now represents the largest white shark on record worldwide (602 cm TL); this shark was measured by a fish factory owner following instructions from one of the authors (see Figure S6.1). Fishermen commonly exaggerate

when estimating the size of large fish (Nakaya 1994, Castro 2012), which may result in a bias in this and other observation data; however, the length-weight data from the Northwest Pacific Ocean (including estimated lengths and weights) agreed with global data recorded by trained scientists providing confidence in the quality of the data. This study reports some of the heaviest white sharks on record and consequently adds important data to the upper end of the length-weight relationship for this species (KwaZulu-Natal Sharks Board, unpublished data) (Fergusson 1996, Francis 1996, Ezcurra et al. 2012).

Single regional nursery grounds for white sharks have long been hypothesized (Domeier 2012a, Dudley 2012), but recent genetic and tracking data indicates the presence of multiple nursery grounds off Eastern Australia (Bruce and Bradford 2012). Similarly, individuals < 200 cm TL in this study were geographically widespread. Assuming that these small juveniles have relatively restricted home ranges (Bruce et al. 2006), our data suggests the occurrence of multiple nursery grounds in the Northwestern Pacific with associated implications for management. White sharks of all sizes were observed throughout the region, suggesting either a lack of size segregation or regionally structured populations. In South Africa, sub-adults and small adults aggregate at pinniped colonies and make offshore migrations (Bonfil et al. 2005, Dudley 2012), while larger adults, including pregnant females, are typically found in the tropical waters of the Western Indian Ocean (Cliff et al. 2000). Similarly in the Northeast Pacific, size segregation occurs and adult sharks aggregate to one of two locations (Central California or Guadalupe Island) and make defined offshore migrations (Domeier 2012a). White sharks have also been documented to use specific habitats by size within aggregation

sites (Robbins and Booth 2012). Accepting that observation data provides only a snap shot of a species' distribution and movement, it is likely, that size segregation occurs in the region but the coarseness of the observation data is unable to determine this.

Conclusions about sexual segregation by season or location could not be made due to under-reporting of sex. Similar to size segregation, it is possible that fine-scale sexual segregation may be occurring within the Northwest Pacific white shark population, but tracking data is required to confirm this. Sexual segregation in white sharks has been described in the Northeast Pacific Ocean, whereby males make annual migrations and large likely pregnant females migrate biannually, remaining in offshore waters for a prolonged period of time (Domeier 2012a, Domeier and Nasby-Lucas 2012). In the Neptune Islands, South Australia, females are only observed from autumn to mid-winter while males are present year round (Bruce et al. 2006). Conversely in False Bay, South Africa, females are present year-round while males are only present during autumn and winter (Kock et al. 2013). Because fishing pressure can affect one sex unequally in sexually segregated populations (Mucientes et al. 2009), it is important to determine the extent of sexual segregation occurring in the region.

Of the 11 pregnant females documented from the Northwest Pacific, four were described previously (Uchida et al. 1987, Nakaya 1994, 1996) and there was one additional suspected pregnant female (Table 6.1). The geographic segregation of these pregnant individuals by gestation stage may suggest that pregnant females have variable habitat preferences during this period. Previous opportunistic sampling of white sharks has allowed the description of the reproductive system in mature males (Pratt 1996) and pregnant females (Uchida et al. 1987, Nakaya 1994, Uchida et al. 1996); however, little is

known about the migration patterns of pregnant females. For the Northeast Pacific, it is thought that females remain offshore during gestation and return to inshore nurseries to pup (Domeier 2012a, Domeier and Nasby-Lucas 2013). This hypothesis is supported by data from other regions, with near-term females being captured nearshore in Kenya (Cliff et al. 2000) and Tunisia (Saïdi et al. 2005). The estimated 20-month gestation period for sharks in the Northwest Pacific is slightly longer than the previously suggested 18-month gestation period (Domeier 2012a, Mollet et al. 2000, Domeier and Nasby-Lucas 2013). The spring (April- June) parturition in the Northwest Pacific, is slightly before the late spring – mid-summer (May-August) parturition found in the Northeastern Pacific (Domeier 2012a), and earlier (assuming offset by 6 months) from the southern hemisphere South Australia parturition, which is purported to occur in summer – mid-autumn (December-May) (Malcolm et al. 2001).

CONCLUSIONS AND RECOMMENDATIONS

White sharks are protected globally by a variety of measures (Table 6.1); however, participation in these agreements varies in the Northwest Pacific. Regional management units may provide a mechanism to preserve genetic diversity and protect distinct population segments (Wallace et al. 2010). This highlights the need for improved regional protection of white sharks. International cooperation among countries throughout the Northwest Pacific is required to establish a management agenda, initiate systematic monitoring and biological sampling programs, and to reach agreement over commitments to international conventions (Barker and Schluessel 2005). Regional workshops that involve various stakeholders (i.e. policy makers, scientists, and local fisherman) should be conducted to determine the best practices to accommodate local

customs and requirements (Techer and Klein 2011). Formal monitoring programs such as the recently enacted regulation in Taiwan requiring fishermen to report catches of white sharks of all size classes (Lee 2013), if properly implemented and enforced, would allow for a more accurate assessment of population trends in the region and help to determine aggregation sites.

There are several broad areas of scientific research that should be conducted to promote conservation (Simpfendorfer et al. 2011). Satellite tagging studies can provide details on migration (by size and sex) (Bonfil et al. 2010, Domeier and Nasby-Lucas 2012), aggregation sites, and potential nursery grounds (Strong et al. 1996, Duffy et al. 2012,), while acoustic tagging can give fine-scale details on localized movements during residency periods (Jorgensen et al. 2012, Kock et al. 2013). Identifying aggregation sites will provide locations to conduct biological sampling to better understand the role of white sharks in regional food webs and continue population assessments. Chemical tracer techniques can be successfully used to infer the diet and trophic ecology of these large predators (Carlisle et al. 2012, Hussey et al. 2012) and hormone analysis, for example, can elucidate information on reproductive status (Awruch et al. 2008). Combining photographic identification with mark recapture or acoustic tracking data can be used to quantitatively investigate population trends (Nasby-Lucas and Domeier 2012, Towner et al. 2013a). In addition, identification of aggregation sites can facilitate economically profitable shark-based ecotourism (Gallagher and Hammerschlag 2011, Vianna et al. 2012). While not without concern over possible harmful effects on ecosystems, white shark cage diving operations at aggregation sites in Australia, Mexico, New Zealand, South Africa, and the US yield financial benefits for operators and associated

communities as well as opportunities for education and research (Gallagher and Hammerschlag 2011).

Mitochondrial DNA analyses of Japanese white sharks indicate these individuals form a monophyletic clade separate from other geographic regions including the Northeast Pacific (Tanaka et al. 2011). Furthermore, of more than 200 white sharks satellite tagged in the Northeast Pacific (Boustany et al. 2002, Dewar et al. 2004, Weng et al. 2007a, b, Domeier and Nasby-Lucas 2008, 2012, 2013, Jorgensen et al. 2010), no animals have yet undertaken trans-oceanic migrations to the Northwest Pacific region. This, coupled with the large number of observations in this study across the entire Northwest Pacific and the relatively stable abundance of animals over the past 60 years, support the occurrence of a distinct sub-population of white sharks, which is widely dispersed from northern temperate to southern tropical latitudes.

REFERENCES

Adams DH, Mitchell ME, Parsons GR (1994) Seasonal occurrence of the white shark, *Carcharodon carcharias,* in the waters off the Florida west coast, with notes on its life history. Mar Fish Rev 56: 24-28

Awruch CA, Frusher SD, Pankhurst NW, Stevens JD (2008) Non-lethal assessment of reproductive characteristics for management and conservation of sharks. Mar Ecol Prog Ser 355: 277-285

Barker MJ, Schluessel V (2005) Managing global shark fisheries: suggestions for prioritizing management strategies. Aquatic Conserv: Mar Freshw Ecosyst 15: 325-347

Blower DC, Pandolfi JM, Bruce BD, Gomez-Cabrera MDC, Ovenden JR (2012) Population genetics of Australian white sharks reveals fine-scale spatial structure, transoceanic dispersal events and low effective population sizes. Mar Ecol Prog Ser 455: 229-244

Bonfil R, Meÿer M, Scholl MC, Johnson R, O'Brien S et al. (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310: 100-103

Bonfil R, Francis MP, Duffy C, Manning MJ, O'Brien S (2010) Large-scale tropical movements and diving behavior of white sharks *Carcharodon carcharias* tagged off New Zealand. Aquat Biol: 8: 115-123

Boustany AM, Davis SF, Pyle P, Anderson SD, Le Boeuf BJ et al. (2002) Expanded niche for white sharks. Nature 415: 35-36

Bruce BD, Bradford RW (2012) Habitat use and spatial dynamics of juvenile white sharks, *Carcharodon carcharias*, in Eastern Australia. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 225-253

Bruce BD, Stevens JD, Malcolm H (2006) Movements and swimming behavior of white sharks (*Carcharodon carcharias*) in Australian waters. Mar Biol 150: 161-172

Bruce BD (1992) Preliminary observations on the biology of the white shark, *Carcharodon carcharias*, in South Australian waters. Aust J Mar Fresh Res 43: 1-11

Camhi MD, Valenti SV, Fordham SV, Fowler SL, Gibson C (2009) The conservation status of pelagic sharks and rays: Report of the IUCN Shark Specialist Group Pelagic Shark Red List Workshop. Newbury: IUCN Species Survival Commission Shark Specialist Group. 78 p.

Carlisle AB, Kim SL, Semmens BX, Madigan DJ, Jorgensen SJ et al. (2012) Using stable isotope analysis to understand the migration and trophic ecology of northeastern Pacific white sharks (*Carcharodon carcharias*). PLoS ONE 7(2): e30492

Castro JI (2012) A summary of observations on the maximum size attained by the white shark, *Carcharodon carcharias*. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 85-90

Cliff G, Dudley SFJ (2011) Reducing the environmental impact of shark-control programs: a case study from KwaZulu-Natal, South Africa. Mar Freshw Res 62: 700-709

Cliff G, Dudley SFJ, Jury MR (1996) Catches of white sharks in KwaZulu-Natal, South Africa and environmental influences. In: Klimley AP, Ainley DG (eds) Great White

Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 351-362
Cliff G, Compagno LJV, Smale MJ, van der Elst RP, Wintner SP (2000) First records of white sharks, *Carcharodon carcharias*, from Mauritius, Zanzibar, Madagascar and Kenya. S Afr J Sci 96: 365-367
Cooke SJ (2008) Biotelemetry and biologging in endangered species research and animal conservation: relevance to regional, national, and IUCN Red List threat assessments. Endanger Species Res 4: 165-185
De Maddalena A, Heim W (2012) Mediterranean great white sharks: a comprehensive study including all recorded sightings. Jefferson: McFarland & Company Inc. 254 p.
Dewar H, Domeier M, Nasby-Lucas N (2004) Insights into young of the year white shark, *Carcharodon carcharias,* behavior in the Southern California Bight. Environ Biol Fish 70: 133-143
Dewar H, Eguchi T, Hyde J, Kinzey D, Kohin S et al. (2013) Status review of the Northeastern Pacific population of white sharks *(Carcharodon carcharias)* under the Endangered Species Act. National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center
Dolganov VN (2012) The capture of a great white shark *Carcharodon carcharias* Linnaeus, 1758 (Carcharodontidae) in Peter the Great Bay (Sea of Japan). Russ J Mar Biol 38: 88-90
Domeier ML (2012a) A new life-history hypothesis for white sharks, *Carcharodon carcharias,* in the Northeastern Pacific. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 199-223
Domeier ML (2012b) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, 543 p.
Domeier M, Nasby-Lucas N (2008) Migration patterns of white sharks *Carcharodon carcharias* tagged at Guadalupe Island, Mexico, and identification of an eastern Pacific shared offshore foraging area. Mar Ecol Prog Ser 370: 221-237
Domeier ML, Nasby-Lucas N (2012) Sex-specific migration patterns and sexual segregation of adult white sharks, *Carcharodon carcharias,* in the Northeastern Pacific. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 133-146
Domeier ML, Nasby-Lucas N (2013) Two-year migration of adult female white sharks (*Carcharodon carcharias*) reveals widely separated nursery areas and conservations concerns. Anim Biotelem 1: 1-10
Dudley SFJ (2012) A review of research on the white shark, *Carcharodon carcharias*, in Southern Africa. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 511-533
Duffy CAJ, Francis MP, Manning MJ, Bonfil R (2012) Regional population connectivity, oceanic habitat, and return migration revealed by satellite tagging of white sharks, *Carcharodon carcharias*, at New Zealand aggregation sites. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 301-318
Dulvy NK, Baum JK, Clark S, Compagno LJV, Cortés E et al. (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays.

Aquat Conserv 18: 459-482
Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM et al. (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3:e00590
Ellis R, McCosker JE (1991) Great white shark. Stanford: Standford University Press 270 p.
Ezcurra JM, Lowe CG, Mollet HF, Ferry LA, O'Sullivan JB (2012) Captive feeding and growth of young-of-the-year white sharks, *Carcharodon carcharias*, at the Monterey Bay Aquarium. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton pp 3-15
Fergusson IK (1996) Distribution and autecology of the white shark in the eastern North Atlantic Ocean and the Mediterranean Sea. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego pp 321-345
Ferretti F, Worm B, Britten GL, Heithaus MR, Lotze HK (2010) Patterns and ecosystem consequences of shark declines in the ocean. Ecol Lett 13: 1055-1071
Francis MP (1996) Observations on a pregnant white shark with a review of reproductive biology. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 157-172
Francis MP, Duffy CAJ, Bonfil R, Manning MJ (2012) The third dimension vertical habitat use by white sharks, *Carcharodon carcharias,* in New Zealand and in Oceanic and tropical waters of the Southwest Pacific Ocean. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 319-342
Gallagher AJ, Hammerschlag N (2011) Global shark currency: the distribution, frequency, and economic value of shark ecotourism. Curr Issues Tourism 14: 797–812
Gubili C, Bilgin R, Kalkan E, Karhan SU, Jones CS et al. (2010) Antipodean white sharks on a Mediterranean walkabout? Historical dispersal leads to genetic discontinuity and an endangered anomalous population. Proc R Soc B 278: 1679-1686
Halpern BS, Selkoe KA, Micheli F, Kappel CV (2007) Evaluating and ranking the vulnerability of global marine ecosystems to anthropogenic threats. Cons Biol 21: 1301-1315
Han JB, Sun FY, Gao XG, He CB, Wang PL et al. (2010) Low microsatellite variation in spotted seal (*Phoca largha*) shows a decrease in population size in the Liaodong Gulf colony. Ann Zool Fennici 47: 15-27
Hussey NE, McCann HM, Cliff G, Dudley SFJ, Wintner SP et al. (2012) Size-based analysis of diet and trophic position of the white shark, *Carcharodon carcharias*, in South African waters. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp 27-49
International White Shark Symposium February 2-6, 2010. Honolulu, Hawaii
Jorgensen SJ, Chapple TK, Anderson S, Hoyos M, Reeb C et al. (2012) Connectivity among white shark coastal aggregation areas in the Northeastern Pacific. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 159-167

Jorgensen SJ, Reeb CA, Chapple TA, Anderson S, Perle C et al. (2010) Philopatry and migration of Pacific white sharks. Proc R Soc B 277: 679-688
Kock A, O'Riain MJ, Mauff K, Meÿer M, Kotze D et al. (2013) Residency, habitat use and sexual segregation of white sharks, *Carcharodon carcharias* in False Bay, South Africa. PLoS ONE 8(1): e55048
Kyne PM, Carlson JK, Ebert DA, Fordham SV, Bizzarro JJ et al. (2012) The Conservation Status of North American, Central American, and Caribbean Chondrichthyans. IUCN Species Survival Commission Shark Specialist Group, Vancouver, Canada. 148 p.
Lee IC (2013) Fisheries Agency's new rule aims to help shark research. Available: http://www.taipeitimes.com/News/taiwan/archives/2013/03/26/2003558045. Accessed 25 April 2013.
Locarnini RA, Mishonov AV, Antonov JI, Boyer TP, Garcia HE et al. (2010) World Ocean Atlas 2009. In: Levitus S (ed) Volume 1: Temperature. Washington DC: NOAA Atlas NESDIS 68, U.S. Government Printing Office 184 p.
Lowe CG, Blasius ME, Jarvis ET, Mason TJ, Goodmanlowe GD et al. (2012) Historic fishery interactions with white sharks in the Southern California Bight. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 169-185
MacNeil MA, McMeans BC, Hussey NE, Vecsei P, Svavarsson J et al. (2012) Biology of the Greenland shark *Somniosus microcephalus*. J Fish Biol 80: 991-1018
Malcolm H, Bruce BD, Stevens JD (2001) A review of the biology and status of white sharks in Australian waters. In: Report to Environment Australia, Marine Species Protection Program. Hobart: CSIRO Marine Research.
Martin RA (2004) Northerly distribution of white sharks, *Carcharodon carcharias*, in the eastern Pacific and relation to ENSO events. Mar Fish Rev 66: 16-26
McPherson JM, Myers RA (2009) How to infer population trends in sparse data: examples with opportunistic sighting records for great white sharks. Diversity Distrib 15: 880-890
Mizuno AW, Suzuki M, Ohtaishi N (2001) Distribution of the spotted seal *Phoca largha* along the coast of Hokkaido, Japan. Mamm Study 26: 109-118
Mollet HF, Cailliet GM, Klimley AP, Ebert DA, Testi AD et al. (1996) A review of length validation methods and protocols to measure large white sharks. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 91-108
Mollet HF, Cliff G, Pratt Jr. HL, Stevens JD (2000) Reproductive biology of the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. Fish Bull 98: 299-318
Moriyasu S (1972) The Tsushima Current. In: Stommel H, Yoshida K (eds) Kuroshio, University of Washington Press, pp 353-369
Mucientes GR, Queiroz N, Sousa LL, Tarroso R, Sims DW (2009) Sexual segregation of pelagic sharks and the potential threat from fisheries. Biol Lett 5: 156-159
Nakaya K (1994) Distribution of white shark in Japanese waters. Fish Sci 60: 515-518
Nakaya K (1996) A fatal shark attack in Aichi Prefecture, Japan, with other confirmed attack cases in Japanese water. Fish Sci 62: 830-831
Nakano H, Nakaya K (1987) Records of the white shark *Carcharodon carcharias* from

Hokkaido, Japan. Jpn J Ichthyol 33: 414-416
Nasby-Lucas N, Domeier ML (2012) Use of photo identification to describe a white shark aggregation at Guadalupe Island, Mexico. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton pp 381-392
Norman JR, Fraser FC (1937) Giant Fishes, Whales and Dolphins. Putnam, London 376 p.
Park KA, Lee EY, Chung SR, Sohn H (2011) Accuracy assessment of sea surface temperature from NOAA/AVHRR data in the seas around Korea and error characteristics. Korean J Remote Sens 27: 663-675
Paterson R (1986) Shark prevention measures working well. Aust Fish 45: 12-18
Payne MC, Brown CA, Reusser DA, Lee H II (2012) Ecoregional analysis of nearshore sea-surface temperature in the North Pacific. PLoS ONE 7(1): e30105
Pratt Jr. HL (1996) Reproduction in the male white shark. In Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 131-138
Robbins RL, Booth DJ (2012) Seasonal sexual and size segregation of white sharks, *Carcharodon carcharias*, in Eastern Australia. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp 287-299
Saïdi B, Bradaï MN, Bouan A, Guélorget O, Capapé C (2005) Capture of a pregnant female white shark, *Carcharodon carcharias* (Lamnidae) in the Gulf of Gabès (southern Tunisia, central Mediterranean) with comments on oophagy in sharks. Cybium 29: 303-307
Santana-Morales O, Sosa-Nishizaki O, Escobedo-Olvera MA, Oñate-González EC, O'Sullivan JB et al. (2012) Incidental catch and ecological observations of juvenile white sharks, *Carcharodon carcharias*, in Western Baja California, Mexico, conservation implications. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 187-198
Simpfendorfer CA, Heupel MR, White WT, Dulvy NK (2011) The importance of research and public opinion to conservation management of sharks and rays: a synthesis. Mar Freshw Res 62: 518-527
Skomal GB, Chisholm J, Correia SJ (2012) Implications of increasing pinniped populations on the diet and abundance of white sharks off the coast of Massachusetts. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 405-417
Smith SE, Au DW, Show C (1998) Intrinsic rebound potentials of 26 species of Pacific sharks. Mar Freshw Res 49: 663-678
Strong Jr. WR, Bruce BD, Murphy RC, Nelson DR (1996) Population dynamics of great white sharks, *Carcharodon carcharias*, in the Spencer Gulf region, South Australia. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 401-414
Tanaka S, Kitamura T, Mochizuki T, Kofuji K (2011) Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kahima-nada, Japan. Mar Freshw Res 62: 548-556

Techera EJ, Klein N (2011) Fragmented governance: reconciling legal strategies for shark conservation and management. Marine Policy 35: 73-78
Towner AV, Wcisel MA, Reisinger RR, Edwards D, Jewell JD (2013a) Gauging the treat: the first population estimate for white sharks in South Africa using photo identification and automated software. PLoS ONE 8(6): e66035
Towner AV, Underhill LG, Jewell OJD, Smale MJ (2013b) Environmental influences on the abundance and sexual composition of white sharks *Carcharodon carcharias* in Gansbaai, South Africa. PLoS ONE 8(8): e71197
Uchida S, Yasuzumi F, Toda M, Okura N (1987) On the observations of reproduction in *Carcharodon carcharias* and *Isurus oxyrinchus*. Jpn Group Elasmobranch Stud Rep 24: 4-6
Uchida S, Todo M, Teshima K, Yano K (1996) Pregnant white sharks and full-term embryos from Japan. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 139-155
Velikanov AY (2010) The record of the white shark *Carcharodon carcharias* (Lamnidae) from Aniva Bay, Sakhalin. J Ichthyol 50: 347-350
Vianna GMS, Meekan MG, Pannell DJ, Marsh SP, Meeuwig JJ (2012) Socio-econmic value and community benefits from shark-diving tourism in Palau: A sustainable use of reef shark populations. Biol Conserv 145: 267-277
Wallace BP, DiMatteo AD, Hurley BJ, Finkbeiner EM, Bolten AB et al. (2010) Regional management units for marine turtles: A novel framework for prioritizing conservation and research across multiple scales. PLoS ONE 5(12): e15465
Wallace BP, DiMatteo AD, Bolten AB, Chaloupka MY, Hutchinson BJ et al. (2011) Global conservation priorities for marine turtles. PLoS ONE 6(9): e24510
Weltz K, Kock AA, Winker H, Attwood C, Sikweyiya M (2013) The influence of environmental variables on the presence of white sharks, *Carcharodon carcharias,* at two popular Cape Town bathing beaches: A generalized additive mixed model. PLoS ONE 8(7): e68554
Weng K, Honebrink R (2013) Occurrence of White Sharks (*Carcharodon carcharias*) in Hawaiian Waters. J Mar Biol doi: 10.1155/2013/598745
Weng KC, O'Sullivan JB, Lowe CG, Winkler CE, Dewar H et al. (2007a) Movements, behavior and habitat preferences of juvenile white sharks *Carcharodon carcharias* in the eastern Pacific. Mar Ecol Prog Ser 338: 211-224
Weng KC, Boustany AM, Pyle P, Anderson SD, Brown A et al. (2007b) Migration and habitat of white sharks (*Carcharodon carcharias*) in the eastern Pacific Ocean. Mar Biol 152(4): 877-894
Wilson SG, Polovina JJ, Stweart BS, Meekan MG (2006) Movements of whale sharks (*Rhincodon typus*) tagged at Ningaloo Reef, Western Australia. Mar Biol 148: 1157-1166

Table 6.1 Participation in international agreements for countries in the northwest Pacific Ocean

Fishing Entity	CMS Party	CMS Shark MoU Signatory	CITES Party	Finning Ban	WCPFC* Member	IOTC* Member
Russia	No	No	Yes	No	No	No
China	No	No	Yes	*	Yes	Yes
Japan	No	No	Yes, Reservation on white shark listing	Yes, with weak standards & some exceptions	Yes	Yes
Philippines	Yes	Yes	Yes	*	Yes	Yes
Vietnam	No	No	Yes	No	No	No
Republic of Korea	No	No	Yes	No	Yes	Yes
Taiwan	No	No	No	Yes, used fin to carcass ratio; now phasing in fins attached rule	Yes	Cannot become member because of lack of membership in UN bodies

*These RFMOs have adopted finning bans (based on a 5% fin to carcass ratio limit). Members are obligated to adopt domestic regulations in line with RFMO measures.

Table 6.2 Description of pregnant white sharks in the Northwest Pacific Ocean and Worldwide

Date	Location	TL (cm)	Weight (kg)	Number of Embryos	Embryo Size (cm TL)	Source
Feb. 16, 1985	Kin, Japan	555	1970	0	egg cases	Uchida et al. 1987, Uchida et al. 1996
Apr. 2, 1986	Taiji, Japan	470	--	7	100 -110	Uchida et al. 1987, Uchida et al. 1996
May 14, 1992	Uchinoura, Japan	480	1500	5	130	Nakaya 1994, Uchida et al. 1996
May 22, 1992	Toyo, Japan	515	2500	10	135-151	Nakaya 1994, Uchida et al. 1996
Mar. 30, 1994	Otuki-Machi, Japan	480	1500	3	140	Author Observation
May 18, 1997	Shiura, Minamijima-machi, Japan	500	1300	5	--	Author Observation
Jan. 18, 2007	Okinawa, Japan	480	1300	7	mid-term	Author Observation
Feb-March 1988	Keelung,Taiwan	--	--	3	100	D. Ebert p.c.
May 1997	Seven Star Lake, Taiwan	670-700	2500		--	Author Observation
Oct. 13, 1997	Baisolian, Taiwan	--	2000	8	--	Author Observation
Nov. 7, 2008	East Taiwan	542	1930		10 and egg cases	Author Observation
Nov. 2, 2012	Taiwan	450	900	6	20	Author Observation
Nov. 13, 1991	North Cape, New Zealand	536	--	7	143-145	Francis 1996
Nov. 17,1981	Queensland, Australia	320*	--	4	--	Paterson 1986
Nov. 26, 1982	Queensland, Australia	400*	--	11	--	Paterson 1986
Nov. 26,1982	Queensland, Australia	420*	--	14	--	Paterson 1986
Mar. 1, 1994	S. Australia	--	--	2	127	JD Stevens p.c.
Nov. 9, 2003	Aaiheke Island New Zealand	--	--	3-5	150	New Zealand Herald
Oct. or Nov.	South Australia	520	--	6-7	30	Bruce 1992
Oct. or Nov.	South Australia	470	--	13	5	Bruce 1992
Oct. or Nov.	South Australia	420	--	11	60	Bruce 1992
Aug. 1, 1976	Skonnet RI,USA	--	--	--	122	Richard Ellis p.c.
July 16, 1996	Malindi, Kenya	--	--	7 - 17	110	Cliff et al. 2000
Summer 1934	Alexandria, Egypt	430	--	9	61	Norman & Fraser 1937, Ellis & McCosker 1991
Sept. 1, 1992	Cape Bon, Tunisia	>500	--	2	Full-term	Fergusson 1996
Feb. 26, 2004	Gulf of Gabes, Southern Tunisia	--	--	4	132-135	Saïdi et al. 2005

p.c.- personal communication

* Francis 1996 suggests incorrect measurements or not TL.

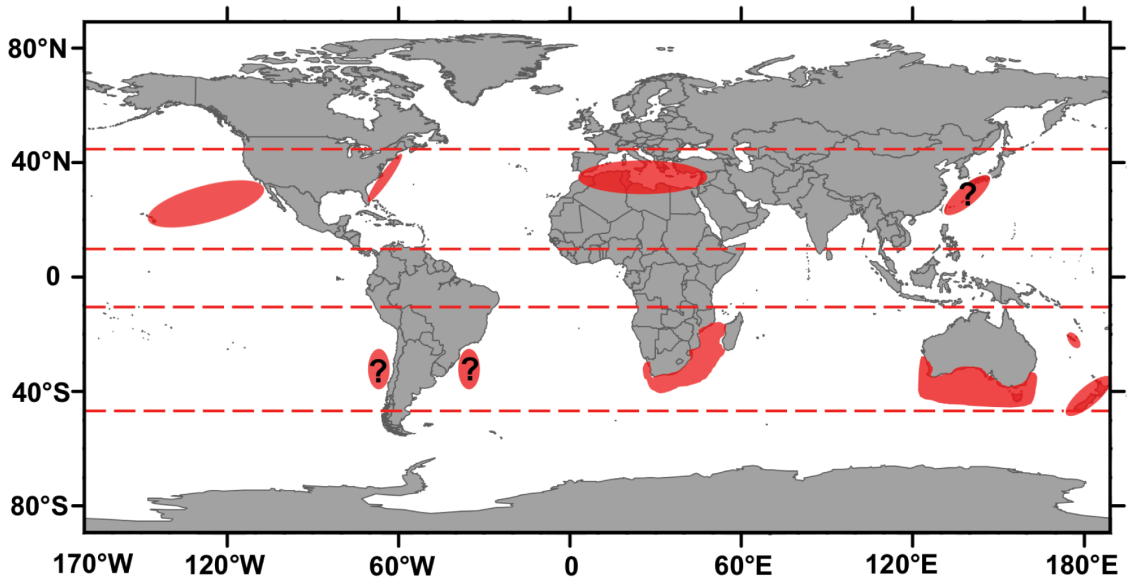

Figure 6.1 Global hotspots of white sharks. Known global white shark aggregation sites. Question marks indicate suitable latitudinal ranges where white sharks may occur, but little is known about current population trends.

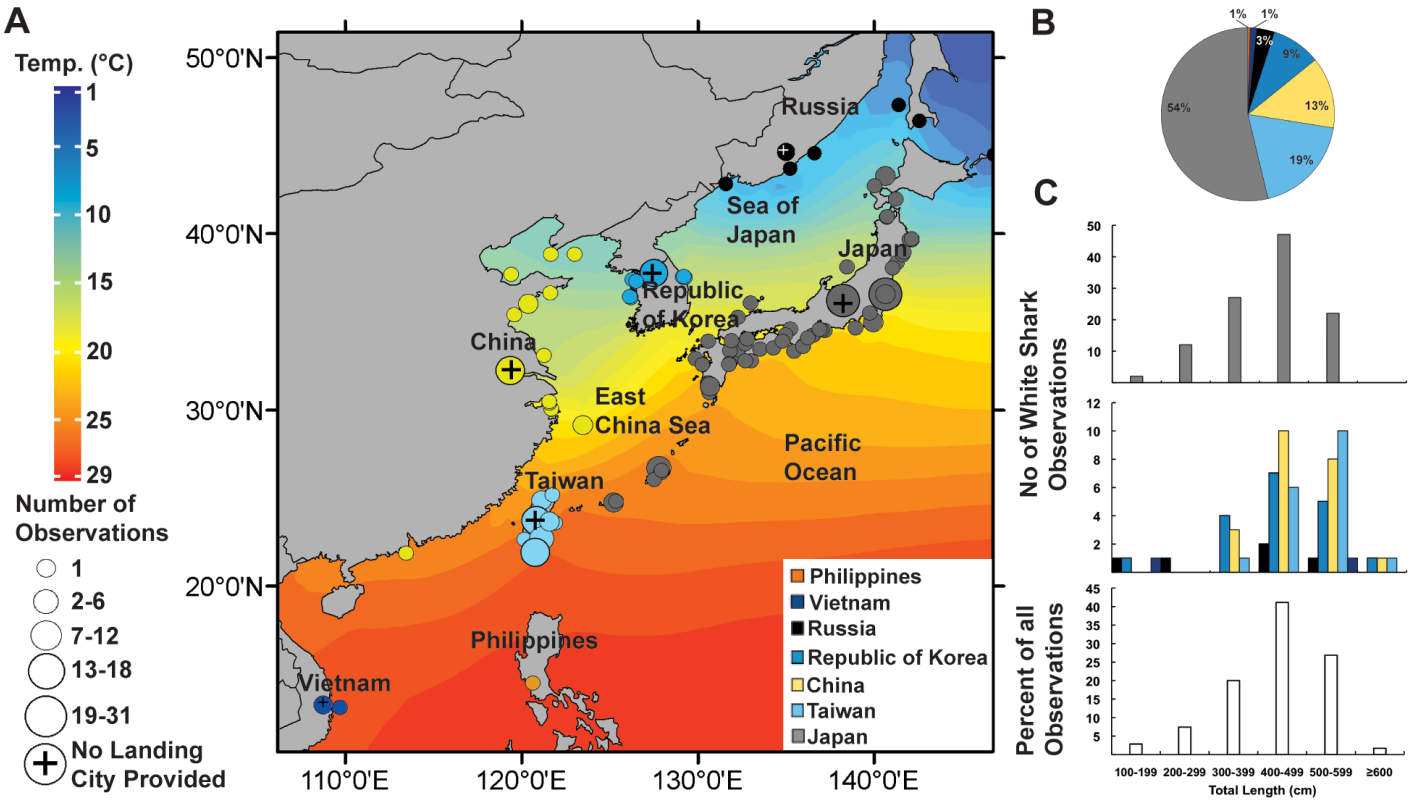

Figure 6.2 Geographical extent and size distribution of white shark observations. Color legend for country of observation occurrence applies to entire figure. **A**- Approximate location of observation or landing for individual white sharks. Circles on land indicate observations that only reported country of landing. Annual average sea surface temperature is indicated by color gradient. **B**- Percentage of white shark observations by country landed. **C**- Size of white shark observed by country landed (top two panels) and as a percent of all observations (bottom panel). Note–the Philippines observations did not have an associated animal size.

Figure 6.3 Male white shark**. A**- Male captured on February 17, 2009 in Taitung, Taiwan measuring 500 cm total length and weighing 1020 kg. Photo obtained from: http://tw.myblog.yahoo.com/jw!duL4dwaTBB7FYwm6Q0vtIm8d/gallery?cfid=144&act=&fid=144&nfid=&yuid=jw!duL4dwaTBB7FYwm6Q0vtIm8d&page=1&.crumb=8mdZj9IAdn6 **B**- Male captured on July 19, 2007 in Aniva Bay Sakhalin, Russia measuring 504 cm total length and weighing 1111kg.

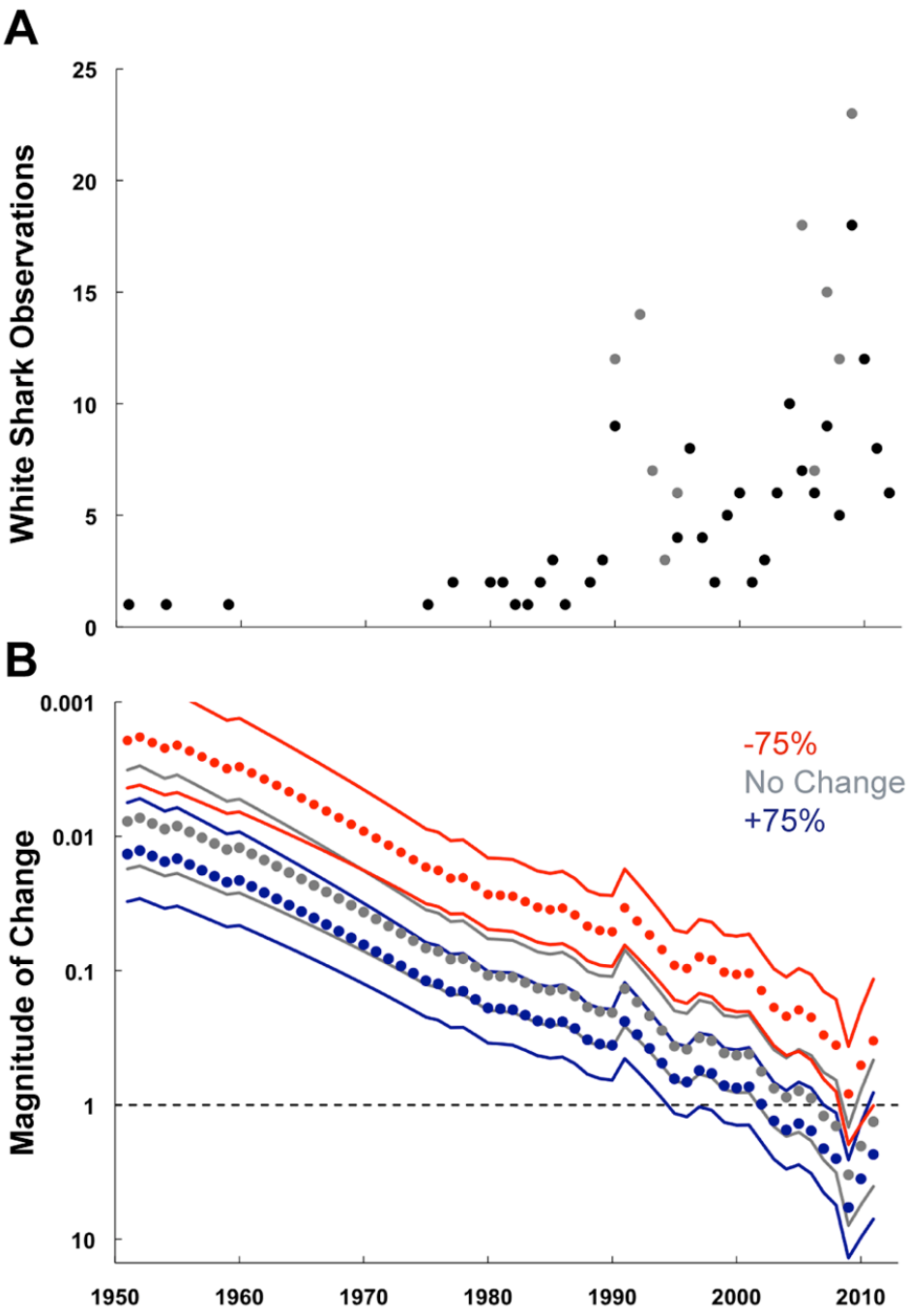

Figure 6.4 Population trend analysis. **A**- White shark observations by year. Black circles indicate data included in population trend analysis. Gray circles indicate data from focused monitoring in Japan that was excluded prior to undertaking the analysis. **B**- Estimates of changes in relative abundance for any reference year between 1951-2011 under different assumptions of trends in observation effort.

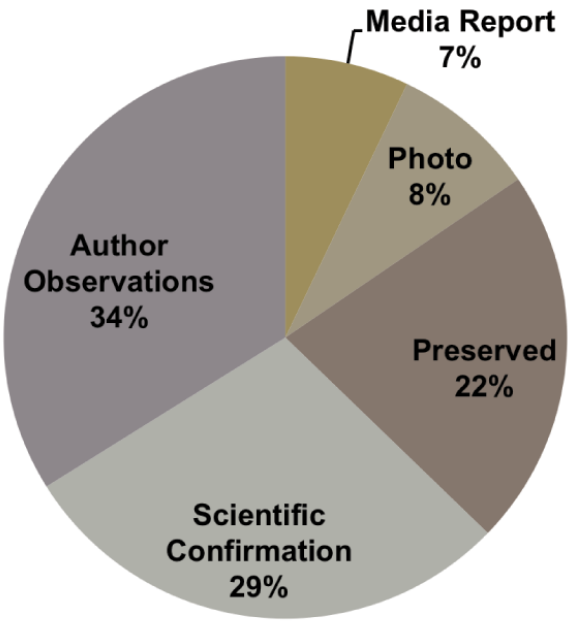

Figure 6.5 Method of confirming white shark observations. Data are shown as a percentage; Media Report-from newspapers or online news sources; Photo-confirmed by pictures of the individual shark (either through personal communications or online but not through news source); Preserved-individuals or remains of an individual, i.e. jaws that are held in a personal or museum collection; Scientific Confirmation-observations that have been previously reported in the scientific literature; Author Observations-personal observations or communications by the authors.

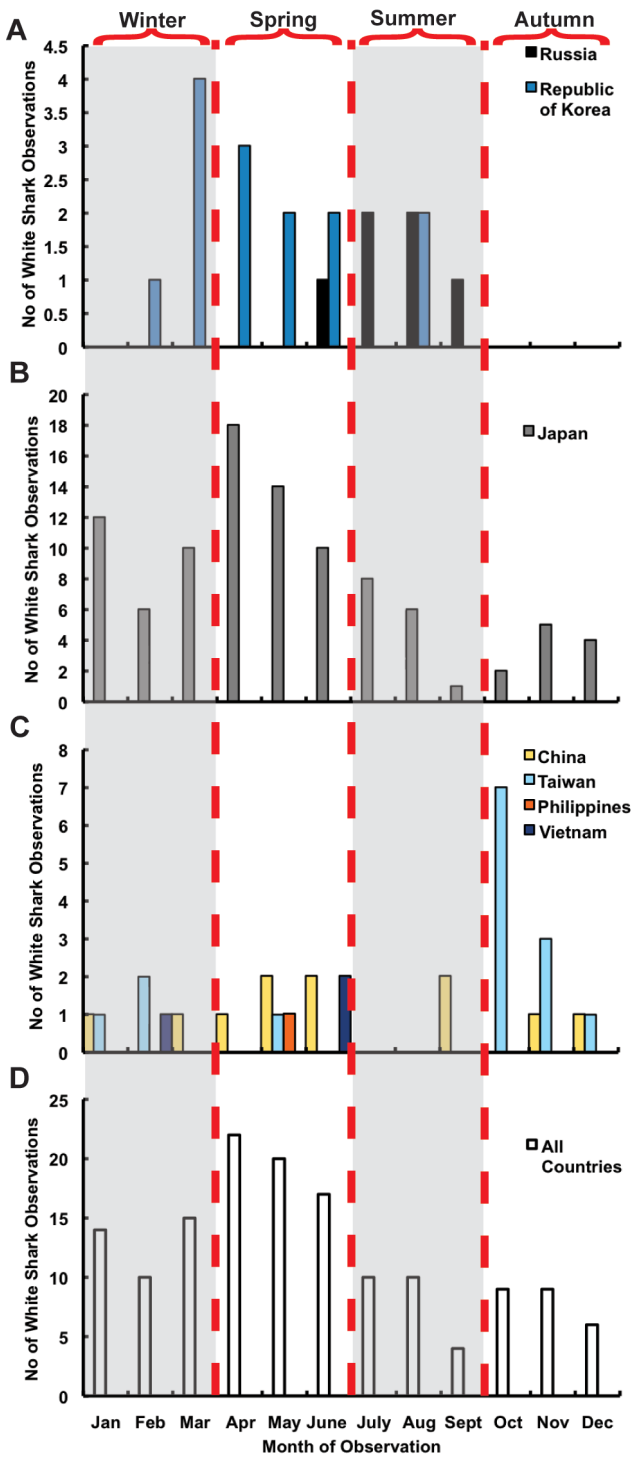

Figure 6.6 White shark observation by month and latitude. **A**- Republic of Korea (blue bars) and Russia (black bars) **B**- Japan (gray bars) **C**- China (yellow bars), Taiwan (light blue bars), Vietnam (dark blue bars), and the Philippines (orange bars), **D**- All observations combined. Winter and summer months are shaded gray.

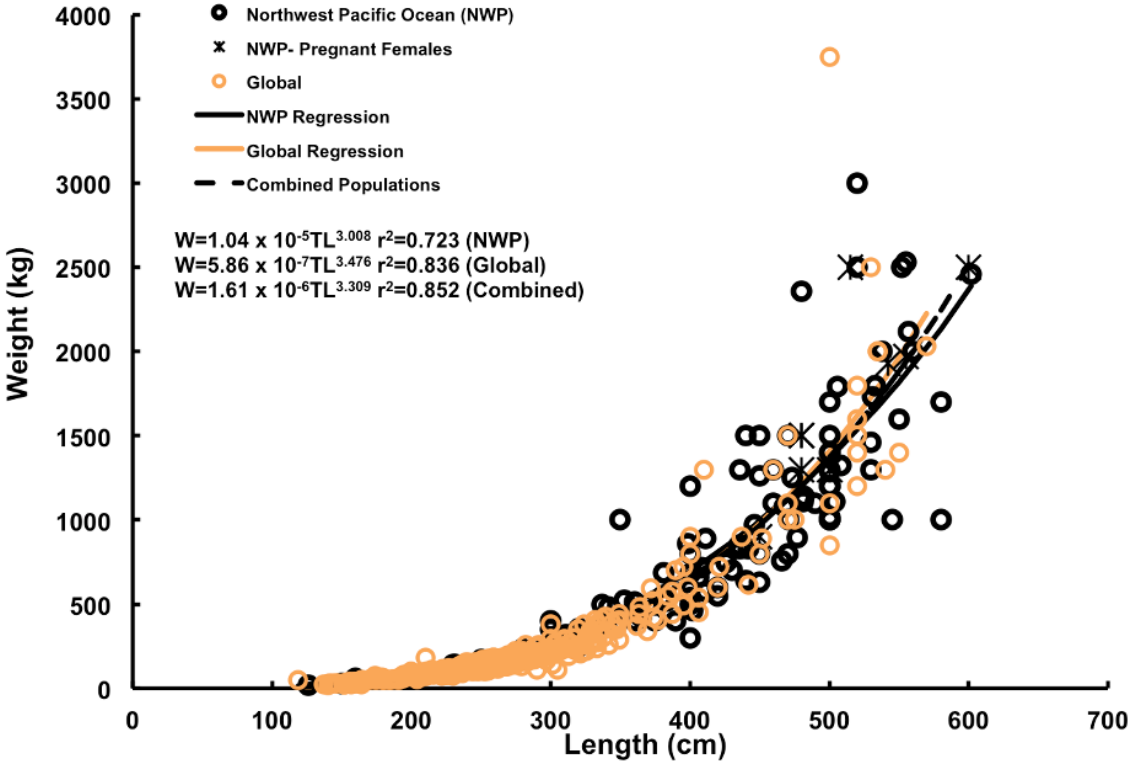

Figure 6.7 Length-weight regressions for white sharks by region. Regressions were fit to white shark data for the Northwest Pacific (black circles/line), global data recorded by trained scientists (orange circles/line) and all data combined (dashed black line). Pregnant females from the Northwest Pacific (black stars) were not included in the analysis.

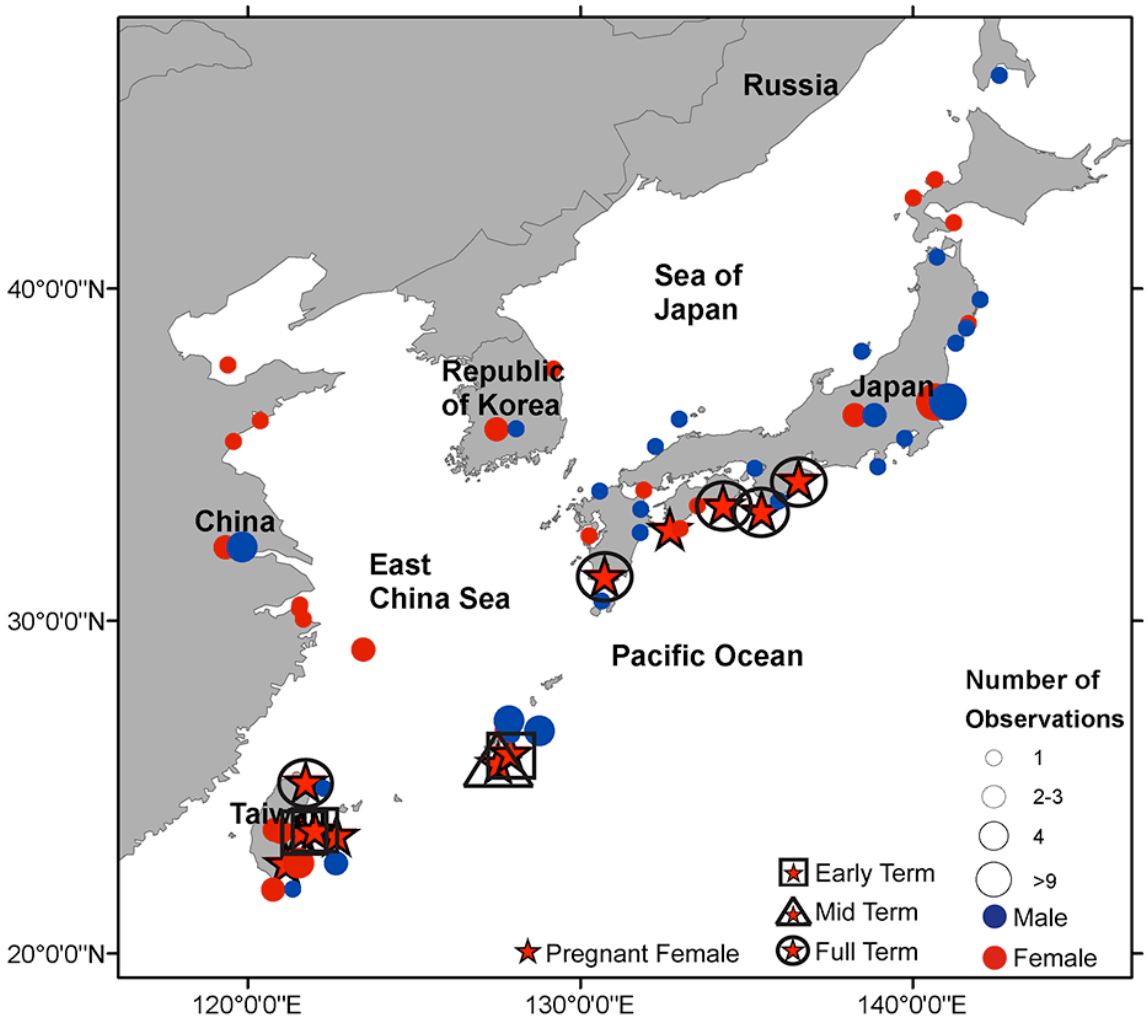

Figure 6.8 Location of white shark observations by sex. The occurrence of male, female and pregnant female white sharks in the Northwest Pacific. Circles on land underneath country label indicate observations that did not report a specific landing location. For pregnant females, black squares indicate early term, black triangles indicate mid term and black circles indicate full term for individuals where embryo size was reported.

A

B

C

Figure 6.9 Pregnant white shark. **A-** Pregnant female captured on November 7, 2008 in Southern Bay Dock, East Taiwan, measuring 542 cm total length and weighing 1930 kg **B**- Ovary **C**- Egg cases measuring 10 cm. Photos obtained from: http://tw.m.wretch.yahoo.com/album/redo0905/12

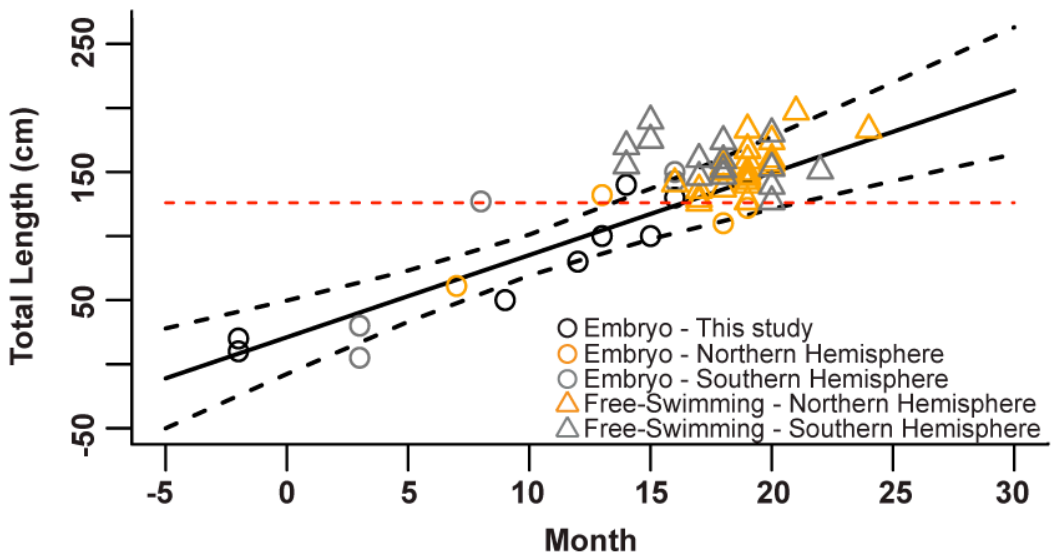

Figure 6.10 Month of observation and size of white shark embryos and free-swimming juveniles worldwide. Data are for white shark observations in this study and globally. Month zero is January in Northern Hemisphere and July in Southern Hemisphere. For unborn animals, black circles indicate embryos from this study (Northwest Pacific; n=9); orange circles indicate embryos from the Northern Hemisphere (n=4), while gray circles indicate embryos from the Southern Hemisphere (n=5). For free-swimming juvenile sharks, orange triangles indicate individuals from the Northern Hemisphere (n=18), and gray triangles indicate individuals from the Southern Hemisphere (n=17). The solid black line indicates the linear regression for embryos from this study, while the dashed black lines indicate the confidence intervals. The red dashed line indicates size at birth.

Table S6.1 Records of White Shark Observations in the Northwest Pacific Ocean 1951-2012

Date	Country	Location of Landing	Latitude	Longitude	Length (cm TL)	Weigh t (kg)	Sex	Fishing Method	Source	Source
	China	TsingTao	36.02	120.39	400*				preserved	Jaws preserved in a museum in TsingTao
1995	China				450.00	1500	M		personal communication	Huang personal communication
1996	China	GuangZhou	21.87	113.46		2950*			preserved	Jaws preserved. Huang personal communication
1999	China					500			media report	Media report
1999	China					2200			personal communication	Huang personal communication
November 1, 2001	China	East Sea	29.15	123.49	580.00	1000*	F		preserved	Mounted in ZheJiang Natural History Museum
September 10, 2002	China	Northern Yellow Sea	38.84	123.02	337.00	500			preserved	Mounted at a local museum in Daliang
2002	China	East Sea	29.15	123.49	602.00	2460	F		personal communication	Author observation
2003	China	TsingTao	36.02	120.39	480.00	2360			personal communication	Author observation
2004	China					500*	F		personal communication	Author observation
2004	China					1400			personal communication	Wen personal communication
2004	China				500*	1700			photo	Photos of being dissembled shown on internet
2005	China	Dalian	38.82	121.66		800			preserved	Mounted for museum display
2005	China	NingPuo (Ningbo)	30.06	121.69	555.00	2530	F		preserved	Author observation
April 18, 2006	China	JaingSu County	33.09	121.29	466.00	755			personal communication	Author observation
2006	China					600			personal communication	Huang personal communication
2007	China	Rizhao, Shandong	35.40	119.59	450.00	630	F		media report	http://english.people.com.cn/200705/09/eng20070509_373174.html

2007	China					1950*	F	personal communication	Author observation
2007	China					800*		personal communication	Li personal communication
2007	China				468.00	1080	M	personal communication	Li personal communication
May 7, 2007	China	ZiZhau, ShanDong	37.70	119.42	450.00	1260	F	media report	Media report
June 12, 2007	China	TsingTao	36.02	120.39	520*	1200*	F	photo	Author observation
March 15, 2008	China	ZhongChen, ShanDong	30.37	121.57		2500	F	personal communication	Author observation
June 10, 2008	China	Zhejiang	30.47	121.59	550.00	1600	F	personal communication	Author observation
2009	China				530.00	1460	M	preserved	Jaws preserved
2009	China				473.00	1250	M	personal communication	Huang personal communication
2009	China					800		personal communication	Li personal communication
2009	China				300*			photo	Photo of mount displayed on street
January 19, 2009	China					600		photo	Photos of being dissembled on street
September 15, 2009	China				460.00	1300		personal communication	Li & Tsai personal communication
May 4, 2010	China	RauShan, ShanDong	36.62	121.63		1500*		photo	Photos of being displayed on street
December 10, 2010	China					1600	F	personal communication	Li personal communication
	Japan							preserved	Mounted at National Museum of Nature and Science
	Japan							preserved	Jaws preserved in unknown museum
	Japan							preserved	Jaws preserved
	Japan							preserved	Jaws preserved in unknown museum

Japan							preserved	Head preserved in Kesennuma Fish Market
Japan							preserved	Mounted at Kanagawa County Aquarium
Japan							preserved	Preserved in Minamichita Aquarium
Japan							preserved	Preserved in Churarumi Aquarium, Okinawa
Japan							preserved	Jaws preserved
Japan							preserved	Preserved head in Himeji-jo Municipal Aquarium
Japan							preserved	Head mounted for display at Oarai mobile aquarium
Japan							preserved	Mounted at Yamahaku County Museum
Japan				150.00			preserved	Jaws preserved
Japan				400.00	540	M	preserved	Mounted at Ibaraki Oarai Aquarium
Japan				400*			preserved	Skin mounted in Ibaraki Oarai Aquarium
Japan				400.0*			preserved	Jaws preserved Churaumi Mobile Aquarium, Okinawa
Japan				420.00			preserved	Mounted at Sakaiminato cultural museum
Japan				450*			preserved	Jaws preserved in Ibaraki Oarai Aquarium
Japan				450*			preserved	Jaws preserved in Ibaraki county Natural Museum
Japan				450*			preserved	Jaws preserved in Hakan Aquarium Okinawa
Japan	Kyushu	32.57	130.27	480.00		F	personal communication	Author observation
Japan				500.00			preserved	Head and fin preserved in Hokkaido University
Japan				500.00	1300	F	preserved	Mold of specimen kept in Rias Shark Museum, Kesennuma
Japan				500.0*	1200*	M	photo	Author observation

	Japan	Aomori	40.95	140.76	500.0*	1000*			preserved	Author observation
	Japan				550*				photo	Author observation
October 2, 1954	Japan	Oomura Bay, Nagasaki	32.90	129.88		2000			media report	K. Nakaya; San Diego Union, 105/1954
November 21, 1975	Japan	Motobu, Okinawa	26.66	127.87	390.00	400	M	set line	scientific confirmation	Uchida et al. 1996
January 18, 1977	Japan	Motobu, Okinawa	26.66	127.87	360.00	400	M	set line	scientific confirmation	Uchida et al. 1996
January 18, 1977	Japan	Motobu, Okinawa	26.66	127.87	400.00	500	M	set line	scientific confirmation	Uchida et al. 1996
March 5, 1980	Japan	Motobu, Okinawa	26.66	127.87	471.00	1000	M	set line	scientific confirmation	Uchida et al. 1996
June 26, 1981	Japan	IE Island, Okinawa	26.70	127.79	506.00	1790	F	set line	scientific confirmation	Uchida et al. 1996
July 9, 1982	Japan	Sainonada, Houjou City,	34.03	132.81	460.00			set net	media report	Asahi News Paper
November 15, 1984	Japan	IE Island, Okinawa	26.70	127.79	400.00	680	M	set line	scientific confirmation	Uchida et al. 1996
February 16, 1985	Japan	Kin, Okinawa	26.44	127.91	555.00	1970	F†		scientific confirmation	Uchida et al. 1996
May 30, 1985	Japan	Todohokke	41.97	141.24	530.00	1300	F	set net	scientific confirmation	Nakano and Nakaya 1987
May 31, 1985	Japan	Furubira	43.27	140.67	580.00	1700	F	set net	scientific confirmation	Nakano and Nakaya 1987
April 2, 1986	Japan	Taiji, Honshu	33.33	135.46	470.00		F†		scientific confirmation	Uchida et al. 1996
Feburary 14, 1988	Japan	Nago Bay, Okinawa	26.57	127.94	380.00	543	M	set line	scientific confirmation	Uchida et al. 1996
August 7, 1989	Japan	IE Island, Okinawa	26.70	127.79	301.00	270	M	set line	scientific confirmation	Uchida et al. 1996
August 15, 1989	Japan	IE Island, Okinawa	26.70	127.79	508.00	1325	F	set line	scientific confirmation	Uchida et al. 1996
August 19, 1989	Japan	IE Island, Okinawa	26.70	127.79	435.00	850	M	set line	scientific confirmation	Uchida et al. 1996

1990	Japan				560.00				personal communication	Nishida personal communication
November 2, 1990	Japan	IE Island, Okinawa	26.70	127.79	436.00	830	F	set line	scientific confirmation	Uchida et al. 1996
November 2, 1990	Japan	IE Island, Okinawa	26.70	127.79	460.00	1100	M	set line	scientific confirmation	Uchida et al. 1996
March 8, 1992	Japan	Matsuyama, Ehime	33.85	132.67					scientific confirmation	Nakaya 1994
March 11, 1992	Japan	Sata, Kagoshima	30.99	130.65	370.00	552*	M	gill net	scientific confirmation	Nakaya 1994
April 18, 1992	Japan	Ashizuri, Kochi	32.77	133.02	375.00	400	F	set net	scientific confirmation	Nakaya 1994
April 21, 1992	Japan	Shimamaki, Hokkaido	42.72	140.02	552.00	2500	F	set net	scientific confirmation	Nakaya 1994
May 14, 1992	Japan	Uchinoura, Kagoshima	31.36	130.74	480.00	1500*	F†	set net	scientific confirmation	Nakaya 1994
May 22, 1992	Japan	Toyo, Kochi	33.51	134.30	515.00	2500	F†	set net	scientific confirmation	Nakaya 1994
May 22, 1992	Japan	Harima-nada, Hyogo	34.59	135.25	490.00	1100	M	trawl	scientific confirmation	Nakaya 1994
May 22, 1992	Japan	Mizunoko Island, Ttsurumi-Machi, Ooita	33.01	132.10	274.00	200*		Purse Seine	personal communication/ photo	Author observation
May 29, 1992	Japan	Furubira, Hokkaido	43.27	140.67	538.00	2000	F	set net	scientific confirmation	Nakaya 1994
June 10, 1992	Japan	Ishinomaki, Miyagi	38.35	141.30	300.00	400	M	set net	scientific confirmation	Nakaya 1994
June 17, 1992	Japan	Taisei, Ikata, Ehime	33.41	132.29	500.00				scientific confirmation	Nakaya 1994
July 7, 1992	Japan	Ugui, Nachi-Katsuura, Wakayama	33.62	135.99	450.00	800	M	set net	scientific confirmation	Nakaya 1994

August 5, 1992	Japan	Nobeoka City, Miyazaki	32.57	131.76	400.00	1200		Bottom trawl net	personal communication	Author observation
December 29, 1992	Japan	Chikura, Chiba	34.98	139.99	570.00			set net	scientific confirmation	Nakaya 1994
January 1993	Japan	Uchinoura, Kagoshima	31.35	130.72	250.00				scientific confirmation	Nakaya 1994
January 4, 1993	Japan	off Namitu, Okagaki-Machi, Fukuoka	33.90	130.59	402.00	460	M	set net	scientific confirmation	Nakaya 1994
January 4, 1993	Japan	Taki, Shimane	35.24	132.28	350.00		M	set net	scientific confirmation	Nakaya 1994
March 4, 1993	Japan	Chikura, Chiba	34.98	139.99	460.00			set net	scientific confirmation	Nakaya 1994
March 17, 1993	Japan	Iyo, Ehime	33.75	132.70					scientific confirmation	Nakaya 1996
April 15, 1993	Japan	Narahara, Oita	33.36	131.82	436.00	1300	M	gill net	scientific confirmation	Nakaya 1994
August 5, 1993	Japan	Nishinoshima, Shimane	36.07	132.99	500.00		M	set net	scientific confirmation	Nakaya 1994
1994	Japan				490.00				personal communication	Shimamoto personal communication
January 4, 1994	Japan	Shimoda, Shizuoka	34.64	138.95	420.00		M	crab basket	scientific confirmation	Nakaya 1994
March 30, 1994	Japan	Otuki-Machi, Hatata, Kouchi	32.78	132.71	480.00	1500	F†	set net	personal communication	Head and two embryos preserved in Suma Aqualife Park
April 9, 1995	Japan	Atsumi-cho, Aichi	34.50	137.21	520.00				scientific confirmation	Nakaya 1996
April 11, 1995	Japan	Ryotsu City, Sado Island	38.10	138.47	420.00	600*	M	set net	personal communication	Author observation
May 28, 1996	Japan	Kamishima, Toba-City, Mie	34.53	137.01	273.00			Purse seine	personal communication	Author observation

July 3, 1996	Japan	Tachikawa, Sumoto City, Hyougo	34.26	134.99	150.00	30		Bottom trawl	personal communication/ photo	Photo
July 7, 1996	Japan	Tubakitomari-Machi, Anan City, Tokushima	33.91	134.80	280.00	200			personal communication/ photo	Photo
July, 1996	Japan	Aise, Ibaraki	36.58	140.66	400.00	540	M	set net	preserved	Dry specimen in Oarai Aquarium
August 1, 1996	Japan	Watari-machi, Miyagi	38.04	141.05	300.00	250*		bottom gill net	personal communication	Author observation
July 23, 1996	Japan	Hirara City, Miyako, Okinawa	24.77	125.23	250.00				media report	M. Shimbun; japanupdate.com
May 18, 1997	Japan	Shiura, Minamijima-machi, Mie	34.25	136.57	500.00	1300	F†	set net	personal communication	Author observation
July 12, 1997	Japan	Hirara City, Miyako, Okinawa	24.77	125.23					media report	Okinawa Weekly Times, 7/19/1997 editon; Ryukyu Shrimpo News (Japan)
December 12, 1998	Japan	Urasiro-machi, Nobeoka, Miyazaki	32.65	131.81	400.00			Set net	personal communication	Author observation
July 8, 1999	Japan	Hikari City, Yamaguchi	33.94	131.92	520*	3000*	F		preserved	Jaws preserved in Kaikyokan Aquarium
July 5, 2000	Japan	Taiji, Wakayama	33.60	135.98	300.00	250	M	Harpoon	personal communication	Author observation
September 16, 2000	Japan	Sunayama Beach, Miyako, Okinawa	24.82	125.37	250*			attack	media report	http://sharkattacksurvivors.com /shark_attack/viewtopic.php?f=7&t=304&p=662&hilit=japan#p662
December 13, 2000	Japan	Aise, Ibaraki	36.58	140.66	400.00		M	set net	personal communication	Author observation

May, 2001	Japan	off Owase, Mie	34.07	136.26	400.00	300		set net	photo	photo
April 14, 2003	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	315.00			set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
June 22, 2003	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	350.00			set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 30, 2004	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	360.00	515	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
May 16, 2004	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	320.00	356	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
June 28, 2004	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	411.00	572	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
November 19, 2004	Japan	Nobeoka, Miyazaki	32.65	131.81	250.00	170	M	set net	scientific confirmation	Kept one day in Ooita aquarium
April 8, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	381.00	684	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 14, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	406.00	663	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 20, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	442.00	828	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 22, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	398.00	861	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 24, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	482.00	1138	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
May 18, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	353.00	525.4	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff

May 27, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	324.00	342	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
June 2, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	383.00	518.5	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
June 8, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	426.00	748	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
June 14, 2005	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	232.00	138.2	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
October 26, 2005	Japan	Chidori Canal, Yako, Kawasaki off	35.48	139.77	481.00	1110	M		photo	Author observation
April 24, 2006	Japan	Houzaura, Minami-Isemachi, Mie	34.24	136.52	320.00	>250		set net	personal communication	Author observation
2007	Japan	Kōchi County	33.45	133.54	530.00		F	set net	personal communication	Author observation
2007	Japan				420.00	550			photo	Photos of jaws posted on blog
2007	Japan				480*				preserved	Jaws on auction
January 18, 2007	Japan	Okinawa	26.04	127.54	480.00	1300	F†		personal communication	Author observation
January 31, 2007	Japan	Kesennuma fish market	38.80	141.62	430.00	700	M		personal communication	Author observation
May 15, 2007	Japan	Kagoshima	31.53	130.57	480.00			set net	preserved	Jaws preserved in unknown museum
2008	Japan				500.00	1000			photo	Photos of jaws posted on blog
2008	Japan	Iwate	39.69	142.13		500*			personal communication	Author observation
February 24, 2008	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	230.00	138.5	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff

March 1, 2008	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	365.00	422.0	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
March 3, 2008	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	231.00	124.2	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 14, 2008	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	280.00	234.0	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
March 27, 2008	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	446.00	970.0	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
January 22, 2009	Japan	Iwate	39.64	142.14		400		set net	personal communication	Author observation
April 20, 2009	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	411.00	889.0	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
April 24, 2009	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	311.00	320.0	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
June 10, 2009	Japan	Rikuzen-Takada, Iwate	38.94	141.69	300.00	350	F	set net	personal communication	Author observation
December 27, 2009	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	408.00	717.5	M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
January 9, 2010	Japan	Aomori fish market	40.94	140.74		800(550 gutted)	M		personal communication	Author observation
March 18, 2010	Japan				300.00		F		personal communication	Author observation
January 28, 2011	Japan	off Heiizaki, Miyako	39.66	142.03	440*	640	M	set net	personal communication	Author observation

February 17, 2011	Japan	Ise Bay, near Kozukumi Island off Toba City, Mie	34.55	136.92	340.00				personal communication	Author observation
April 26, 2011	Japan				400*				personal communication	Author observation
January 27, 2012	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	360.00		M	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
February 1, 2012	Japan	Kashima-nada off Aise, Ibaraki	36.58	140.66	342.00	480	F	set net	scientific confirmation	Measurement by Ibaraki Prefectural Oarai Aquarium staff
February 1, 2012	Japan	Kagoshima fish market	31.52	130.63	250*				photo	Author observation
May 1, 2003	Philippines	Limay, Baatan	14.52	120.63					media report	D. Cervantes, Star http://www.philstar.com/nation/207333/man-eating-white-sharks-sighted-bataan
June 9, 1951	Russia	Tatar Strait, Sakhalin	47.32	141.43	477	894			scientific confirmation	Dolganov 2012, Velikanov 2010, Probatov 1952
1984	Russia	Cape Gamov			200				scientific confirmation	Dolganov 2012
1999	Russia	Primoriye, Olga Bay	43.69	135.25	400				personal communication	Author observation
July 30, 2004	Russia	Kunahir Island	44.49	146.84					media report	M. Gozum & J. Eager, scubaradio.com
July 19, 2007	Russia	Aniva Bay, Sakhalin	46.41	142.61	504.00	1111	M	seine	scientific confirmation	Velikanov 2010
September 25, 2011	Russia	Primorye, Popov Island			126.00	16			scientific confirmation	Dolganov 2012
August 17, 2011	Russia	Primosky Krai	44.57	136.64					scientific confirmation/ media	http://www.nytimes.com/2011/08/19/world/europe/19jaws.html

August 18, 2011	Russia	Zheltukhina Island	42.82	131.61				media report	http://www.huffingtonpost.com/2011/08/19/two-injured-in-unpreceden_n_931156.html
	Republic of Korea				350*			personal communication	Author observation
Summer 1959	Republic of Korea		37.28	126.52				personal communication	Y. Choi & K. Nakaya
May 23, 1981	Republic of Korea	Wae-yeon Island Chungman	36.35	126.19	600.00			personal communication	Y. Choi & K. Nakaya
June 1996	Republic of Korea				480.00		M	preserved	Mounted at Busan Marine Natural History Museum
May 1, 1998	Republic of Korea				500*			personal communication	Author observation
April 14, 2000	Republic of Korea							personal communication	Author observation
April 25, 2005	Republic of Korea					1500	F	personal communication	Author observation
June 14, 2005	Republic of Korea	Kaeui Island, South Chungchong	36.42	126.15	300.00			media report	http://sharkattackmonitor.wordpress.com/2005/06/22/injured-14-june-2005-kaeui-island-korea-diver-attacked/
April 4, 2007	Republic of Korea				460.00			personal communication	Author observation
February 1, 2009	Republic of Korea	Mukho	37.52	129.27	350.00	2000		media report	http://www.koreatimes.co.kr/www/news/nation/2009/07/117_47190.html
March 1, 2009	Republic of Korea	Mukho	37.52	129.27	470.00	1360*		media report	http://www.koreatimes.co.kr/www/news/nation/2009/07/117_47190.html
March 6, 2009	Republic of Korea				500*			photo	Photos of being dissembled shown on internet
March 9, 2009	Republic of Korea				440.00			personal communication	Author observation
March 27, 2009	Republic of Korea	Mukhohang	37.57	129.21	440*	1500	F	personal communication	Author observation

August 10, 2009	Republic of Korea	Eojongin			545.00	1000	F	personal communication	Author observation
August 10, 2009	Republic of Korea	Incheon	37.38	126.30	470.00	800		personal communication	Author observation
2010	Republic of Korea							photo	Photos of being dissembled shown on internet
2010	Republic of Korea				130.00			personal communication	Author observation
2010	Republic of Korea				360*			photo	Author observation
2010	Republic of Korea				400*			photo	Author observation
2010	Republic of Korea				500*	1200*		photo	Photos of being dissembled shown on internet
2011	Republic of Korea	Donghae	37.53	129.16	560*	2000*		photo	TL and mass estimated from the photo
~1980	Taiwan					2100		personal communication	Li personal communication. Teeth preserved.
1983	Taiwan				400*			preserved	Captured by Taiwanese fishing boat. Jaws shown on Japanese website
Feb-March 1988	Taiwan	Keelung	25.18	121.74		3000*	F†	personal communication	Dave Ebert personal communication
1990	Taiwan	ChenKung, Taitung	22.72	121.16		700		preserved	Jaws preserved in local museum
1990	Taiwan	Southern Bay	21.94	120.77		809		personal communication	Chang personal communication
1990	Taiwan	Southern Bay	21.94	120.77		1000		personal communication	Zhuang personal communication
1990	Taiwan	Southern Bay	21.94	120.77		1000	M	personal communication	Huang personal communication
1990	Taiwan	ChenKung, Taitung	22.72	121.16		1100		preserved	Jaws preserved in local museum
1990	Taiwan	Southern Bay	21.94	120.77		1200*		personal communication	Chen personal communication

1990	Taiwan	Southern Bay	21.94	120.77		1200		personal communication	Hsu personal communication
1990	Taiwan	ChenKung, Taitung	22.72	121.16		1300	F	preserved	Jaws preserved in local museum
1990	Taiwan	Southern Bay	21.94	120.77		2050*		personal communication	Author observation
1995	Taiwan				400*			preserved	Teeth preserved
1995	Taiwan				400*	800		preserved	Jaws preserved for sale
October 1995	Taiwan	ChenKung, Taitung	22.72	121.16	500*	1400	F	preserved	Whole specimen in local museum
May 1997	Taiwan	Seven Star Lake	23.67	121.61	670*	2500	F††	preserved	Jaws preserved in local museum.
October 13, 1997	Taiwan	Baisolian, Taitung	22.72	121.16		2000	F†	preserved	Jaws, fins, vertebrae and embryos preserved
1999	Taiwan	ChenKung, Taitung	22.72	121.16		1500	F	preserved	Jaws preserved in local museum
October 17, 2000	Taiwan	Southern Bay	21.94	120.77	340.00	367	F	preserved	Jaws preserved
October 23, 2000	Taiwan	SanShenTai, Taitung	22.72	121.16	500*	1200	M	personal communication	Author observation
2002	Taiwan	Southern Bay	21.94	120.77		720	F	personal communication	Author observation
2003	Taiwan	Seven Star Lake	23.67	121.61		810*		preserved	Jaws preserved, Shou personal communication
2003	Taiwan	Southern Bay	21.94	120.77		130		personal communication	Author observation
January 2, 2004	Taiwan	S.E. Taiwan	22.36	121.13	557*	2120		preserved	Vertebrae preserved
December 17, 2004	Taiwan	ChenKung, Taitung	22.72	121.16	500*	1400*		photo	Brought to ChenKung fish market
2005	Taiwan	Southern Bay	21.94	120.77		400		personal communication	Author observation
October 13, 2005	Taiwan	Seven Star Lake	23.67	121.61	531.00	1730*	F	media report	Author observation
2005	Taiwan	Kauhsaung	22.65	120.16		2000		preserved	Jaws preserved.
2006	Taiwan	Southern Bay	21.94	120.77		53	F	personal communication	Author observation

2006	Taiwan	Seven Star Lake	23.67	121.61		425			personal communication	Author observation
2006	Taiwan	Seven Star Lake	23.67	121.61	400*	590	F		personal communication	Author observation
2007	Taiwan					800			preserved	Jaws put on auction
2008	Taiwan	DaLi, I-Lan	24.96	121.92		800	M		personal communication	Author observation
2008	Taiwan	Southern Bay brought to Southern	21.94	120.77		107			personal communication	Author observation
November 7, 2008	Taiwan	Bay Dock, East Taiwan	23.57	121.91	542.00	1930	F†		personal communication	Author observation
February 17, 2009	Taiwan	Taitung	22.72	121.16	500*	1020	M		personal communication	Author observation
2009	Taiwan					209			preserved	Author observation
2009	Taiwan	DaShi, I-Lan	24.89	121.16		130			preserved	Jaws preserved
October 20, 2009	Taiwan	Baisolian, Taitung	22.72	121.16	533.00	1800*	F		photo	Author observation
2010	Taiwan	Southern Bay	21.94	120.77		700*			personal communication	Wang personal communication
2010	Taiwan	ChenKung, Taitung	22.72	121.16		800*			personal communication	Author observation
October 27, 2012	Taiwan	Northeast			500*	1500*	F	set net	Personal communication	Author observation
November 2, 2012	Taiwan				500.00	1286	F		personal communication	Author observation
November 2, 2012	Taiwan				450.00	900	F†		personal communication	Author observation
June 14, 2009	Vietnam								media report	Tin Tuc online, 6/14/2009
February 4, 2010	Vietnam	Quy Nhon City	13.12	109.69	500.00	1000			personal communication	Luis Kmentt and Helmut Nickel personal communication
June 1, 2011	Vietnam	Qui Nhon Bay Binh Dinh			1.60	60		set net	media report	http://travelweekly.asia/news/tourism/3689-shark-scare-at-vietnam-resort

* Estimated length or weight based on photo or calculated based on jaw measurements
† Pregnant female
†† Suspected pregnant female

References

Dolganov VN (2012) The capture of a great white shark *Carcharodon carcharias* Linnaeus, 1758 (Carcharodontidae) in Peter the Great Bay (Sea of Japan). Russ J Mar Biol 38: 88-90
Nakano H, Nakaya K (1987) Records of the white shark *Carcharodon carcharias* from Hokkaido, Japan. Jpn J Ichthyol 33: 414-416
Nakaya K (1994) Distribution of white shark in Japanese waters. Fish Sci 60: 515-518
Nakaya K (1996) A fatal shark attack in Aichi Prefecture, Japan, with other confirmed attack cases in Japanese water. Fish Sci 62: 830-831
Probatov AN (1952) A Shark at the Coast of Sakhalin, Priroda 6: 115
Uchida S, Todo M, Teshima K, Yano K (1996) Pregnant white sharks and full-term embryos from Japan. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 139-155
Velikanov AY (2010) The record of the white shark *Carcharodon carcharias* (Lamnidae) from Aniva Bay, Sakhalin. J Ichthyol 50: 347-350

Table S6.2 Global records of white shark embryos and free-swimming juveniles

Date of Capture	Location	Total Length (cm)	Stage	Source
Aug 1, 1976	Rhode Island, USA	122	Embryo	R. Ellis pc
July 16, 1996	Malindi Kenya	110	Embryo	Cliff et al. 2000
Summer 1934	Alexandria, Egypt	61	Embryo	Norman & Fraser 1937, Ellis and McCosker 1991
Feb 26, 2004	Gulf of Gabes, southern Tunisia	132	Embryo	Saïdi et al. 2005
Sept 1936	California, USA	167.6	Free-swimming	Cailliet et al. 1985
Apr 18, 1974	North Carolina, USA	197	Free-swimming	G. Burgess p.c.
Aug 1975	Rhode Island, USA	127	Free-swimming	Ellis and McCosker 1991
July 1976	California, USA	136	Free-swimming	Cailliet et al. 1985
July 1976	California, USA	150	Free-swimming	Cailliet et al. 1985
Oct 1979	California, USA	183	Free-swimming	Cailliet et al. 1985
Aug 1980	California, USA	146	Free-swimming	Cailliet et al. 1985
June 1981	California, USA	129	Free-swimming	Cailliet et al. 1985
Aug 1981	California, USA	147	Free-swimming	Cailliet et al. 1985
Aug 3, 1981	California, USA	160	Free-swimming	Ellis and McCosker 1991
Sept 1981	California, USA	159	Free-swimming	Cailliet et al. 1985
Aug 1983	California, USA	167	Free-swimming	Cailliet et al. 1985
July 1, 1984	California, USA	150	Free-swimming	Hewitt 1984
Sept 10, 1984	California, USA	154	Free-swimming	Mollet et al. 1996
Sept 10, 1984	California, USA	154	Free-swimming	Ellis and McCosker 1991
June 18, 1985	California, USA	126	Free-swimming	J. Seigel p.c.
Jan 6, 1994	Georgia, USA	183	Free-swimming	G. Burgess p.c.
May 30, 2000	California, USA	140	Free-swimming	Dewar et al. 2004
Aug 20, 2004	California, USA	141	Free-swimming	Ezcurra et al. 2012
Aug 17, 2006	California, USA	174	Free-swimming	Ezcurra et al. 2012
Aug 4, 2007	California, USA	143	Free-swimming	Ezcurra et al. 2012
Aug 16, 2008	California, USA	137	Free-swimming	Ezcurra et al. 2012
Aug 13, 2009	California, USA	157	Free-swimming	Ezcurra et al. 2012
Nov 13, 1991	North Cape, New Zealand	143	Embryo	Francis 1996
Nov 9, 2003	Waiheke Island, New Zealand	150	Embryo	New Zealand Herald
Oct or Nov	West Coast Australia	30	Embryo	Bruce 1992
Oct or Nov	West Coast Australia	5	Embryo	Bruce 1992
Mar 1, 1994	South Australia	127	Embryo	JD Stevens p.c.
Sept 27, 1964	South Africa	170	Free-swimming	Francis 1996
Mar 1, 1967	NSW Australia	139	Free-swimming	Francis 1996
Mar 1, 1981	NSW Australia	153	Free-swimming	Francis 1996
Dec 1, 1984	NSW Australia	146	Free-swimming	Francis 1996
Jan 1, 1985	South Africa	159	Free-swimming	Francis 1996
May 1, 1986	South Africa	151	Free-swimming	Francis 1996
Dec 15, 1989	Eastern Cape, South Africa	160	Free-swimming	Francis 1996
Dec 15, 1989	South Africa	160	Free-swimming	Francis 1996
Sept 14, 1991	New Zealand	155	Free-swimming	Francis 1996
Jan 15, 1992	New Zealand	174	Free-swimming	Francis 1996
Jan 18, 1992	NSW Australia	147	Free-swimming	Francis 1996
Jan 19, 1993	New Zealand	152	Free-swimming	Francis 1996
Mar 1, 2000	Australia	180	Free-swimming	Bruce and Bradford 2012
Oct 8, 2007	Australia	190	Free-swimming	Bruce and Bradford 2012
Oct 11, 2007	Australia	190	Free-swimming	Bruce and Bradford 2012
Oct 28, 2008	Australia	175	Free-swimming	Bruce and Bradford 2012

p.c. –personal communication

REFERENCES

Bruce BD (1992) Preliminary observations on the biology of the white shark, *Carcharodon carcharias*, in South Australian waters. Aust J Mar Fresh Res 43: 1-11

Bruce BD, Bradford RW (2012) Habitat use and spatial dynamics of juvenile white sharks, *Carcharodon carcharias*, in Eastern Australia. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 225-253

Cailliet GM, Natanson LJ, Welden BA, Ebert DA (1985) Preliminary studies on the age and growth of the white shark, *Carcharodon carcharias,* using vertebral bands. Mem South Calif Acad Sci 9: 49–60

Cliff G, Compagno LJV, Smale MJ, van der Elst RP, Wintner SP (2000) First records of white sharks, *Carcharodon carcharias*, from Mauritius, Zanzibar, Madagascar and Kenya. S Afr J Sci 96: 365-367

Dewar H, Domeier M, Nasby-Lucas N (2004) Insights into young of the year white shark, *Carcharodon carcharias,* behavior in the Southern California Bight. Environ Biol Fish 70: 133-143

Ellis R, McCosker JE (1991) Great white shark. Stanford: Standford University Press 270 p.

Hewitt JC (1984) The great white shark in captivity: a history and prognosis. AAZPA Annual Proceedings pp. 317-324

Ezcurra JM, Lowe CG, Mollet HF, Ferry LA, O'Sullivan JB (2012) Captive feeding and growth of young-of-the-year white sharks, *Carcharodon carcharias*, at the Monterey Bay Aquarium. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 3-15

Francis MP (1996) Observations on a pregnant white shark with a review of reproductive biology. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 157-172

Mollet HF, Cailliet GM, Klimley AP, Ebert DA, Testi AD et al. (1996) A review of length validation methods and protocols to measure large white sharks. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 91-108

Norman JR, Fraser FC (1937) Giant Fishes, Whales and Dolphins. Putnam, London 376 p.

Saïdi B, Bradaï MN, Bouan A, Guélorget O, Capapé C (2005) Capture of a pregnant female white shark, *Carcharodon carcharias* (Lamnidae) in the Gulf of Gabès (southern Tunisia, central Mediterranean) with comments on oophagy in sharks. Cybium 29: 303-307

Figure S6.1 White shark total length measuring protocol.

CHAPTER 7: GENERAL DISCUSSION

As management and conservation moves from focusing on individual species towards an ecosystem-based approach, it is important to examine the interactions top predators have with their ecosystems (Lester et al. 2010). The degree in which the removal of apex predators will affect the surrounding ecosystem is difficult to predict, however for species that have common aggregation sites it is likely there would be a localized effect of their removal on prey species. Interactions among species are often complex (Hussey et al. 2015) and data deficiencies in the key characteristics in the spatial ecology and life-history traits of threated species hinder our ability to create ecosystem wide management and conservation policies (Simpfendorfer et al. 2011, Dulvy et al. 2014). Implications of using inaccurate parameters when conducting stock assessments can be detrimental to a species (Lai and Gunderson 1987, Cailliet and Andrews 2008) however, managers are often faced with creating management plans and stock assessments based on limited data (Walker et al. 1998, Cortés et al. 2012). This dissertation presents methods that advance our ability to study these key characteristics in a threatened species. Furthermore, through the use of these techniques this dissertation provides information critical to the continued management of a threatened mobile marine predator.

CONTRIBUTIONS TO SCIENCE

Advancing methods to study threatened species

An underlying component to this dissertation was the methods used to study threatened species. Stable isotopes are a powerful tool that can elucidate data on habitat, migration, and trophic ecology (Hobson 1999, Post 2002, Rubenstein and Hobson 2004,

Boecklen et al. 2011). However, several assumptions are typically made when using chemical tracers and the need for laboratory and controlled studies to verify these assumptions has been recognized (Gannes et al. 1997). Elasmobranch vertebrae are biomineralized structures containing both an organic portion and an inorganic portion ((Dean and Summers 2006, Porter et al. 2006, Zhang et al. 2009). The prevailing paradigm in elasmobranch literature was that demineralization of vertebral samples is required prior to stable isotope analysis to remove any bias from the inorganic component (Kim and Koch 2012). The demineralization process requires a large amount of starting sample material therefore reducing ability to serially sample vertebrae at small temporal scales. Data provided by this dissertation in Chapter 2 refuted the assumption that the inorganic component would bias bulk stable isotope values in three species of sharks. This chapter also highlighted the importance of a species-specific minimum sample weight to obtain consistent and reliable data. While Chapter 2 provided information required for the stable isotope analysis conducted in Chapters 4 and 5, its broader significance can be applied to any stable isotope study using elasmobranch vertebrae. The utility of elasmobranch vertebrae to provide unique insight in to retrospective characteristics of a species has been recognized (Kim et al. 2012, Carlisle et al. 2014) and this chapter tests key assumptions of using this method in elasmobranch vertebrae.

Addressing data deficiencies

In this dissertation life-history characteristics and details on the spatial ecology for two populations of white sharks were investigated. For white sharks off southern Africa chemical tracer analysis indicated white sharks are longer-lived and slower growing than previously thought. Additionally, both juveniles and sub-adults have

variable stable isotope profiles indicating divergent life-history strategies within these stages. Based on stable isotope values and observation records it was determined that in both populations, juveniles and sub-adults used a wide range of habitat including tropical and temperate waters. A variety of policies are available to promote the conservation and management of shark species (Shiffman and Hammerschlag 2016) and these data will help guide national and international regulations in both the southwest Indian Ocean and northwest Pacific Ocean. White sharks are protected through various international agreements (Chapter 6); however further protections could be extended to white sharks through the use of regional fisheries management organizations (Wallace et al. 2010).

Accurate life-history parameters (e.g., age and growth, reproductive details) are required to model demographics and create stock assessment models to assess population status to establish if a sustainable fishery is possible for a species (Fig 7.1)(Cortés et al 2012, Beamish et al. 2006, Cailliet and Andrews 2008). In this dissertation annual growth band formation in white sharks off Southern Africa was confirmed for individuals up to age 38 (Chapter 3). Accepting the small number of mature individuals in this study, age-at-maturity was determined to be higher than previous studies estimated for this region (Wintner and Cliff 1999). Populations that have later ages at maturity typically have slower turnover times and a decreased ability to quickly recover from increased fishing pressures (Smith et al. 1998, Goldman et al. 2012). Through population modeling it was found that white sharks had a low rebound potential when the age of maturity was estimated at 9 years old (Smith et al. 1998), therefore the increase in age-at maturity documented in Chapter 3 is likely to decrease the rebound potential even further. The gestation length (20 months) and small litter size (6) for white sharks found in this study

(Chapter 6) support the previously suggested low reproductive potential for this species. These details support the species-specific fisheries ban enacted by individual countries worldwide (Chapter 6) and promote the requirement for further international cooperation to reduce direct fishing and bycatch of white sharks.

Understanding individual specialization in the diet of top predators can provide details on trophic relationships within an ecosystem (Fig 7.1) (Hückstädt et al. 2011). Juvenile white sharks were found to potentially display individual specialization within a generalist population (Chapter 4), while sub-adults were determined to have generalist-feeding strategies (Chapter 5). Examining the diet of white sharks can help predict ecological responses to any reduction in white shark populations and the implications for the ecosystem.

To apply the appropriate scale of management (i.e., state, regional, national, or international) details on the spatial ecology of a species are required (Fig 7.1). By identifying habitat that is important over the course of an individuals lifetime (e.g. nursery, breeding or foraging grounds) the effectiveness of different management strategies such as marine protected areas or shark sanctuaries can be evaluated (Knip et al. 2012). Stable isotope analyses of the juvenile life-stage revealed high inter-individual variation among white sharks indicating juvenile white sharks undertook a wide variety of ecological strategies and are likely widely distributed throughout the region (Chapter 4). By using prey contribution to an individual as a proxy for habitat, it was revealed that sub-adult white sharks rely on habitat outside the boundaries of current management in South Africa (Chapter 5). Similarly, white sharks in the northwest Pacific Ocean were widespread and accepting limited sample size individuals were not segregated by size or

sex. These details indicate further international management is required to protect white sharks at all life-stages in both the southwest Indian Ocean and northwest Pacific Ocean.

FUTURE WORK

While this dissertation provided details on stage specific characteristics, there is still much to learn before fully understanding the spatial and foraging ecology of white sharks. Additionally, the methods outlined above can also be applied to other elasmobranch species for which large collections of vertebrae are available. Furthermore, these techniques are applicable to a wide range of species that have biomineralized structures including coral, otoliths, vibrissae, baleen, and teeth. Perhaps the greatest potential lies in integrating stable isotope analysis with other techniques such as genetic analysis and telemetry data to provide further details on key characteristics of threatened species.

Genetic differences have been described for the white shark worldwide (Pardini et al. 2001, Tanaka et al. 2011, Gubili et al. 2010, O'Leary et al. 2015). However, Andreotti et al. (2015) found no evidence of significant population differences among aggregation sites and failed to find sub-structure within the South African population. Further work to investigate whether genetic sub-structure of white sharks in South Africa should be undertaken. If there is population sub-structures these genetic differences can be compared with stable isotope values to further examine if genetically similar individuals are utilizing the same habitat. Additionally, identifying if genetically separated nurseries are occurring is an important component to creating a comprehensive management plan (Kinney and Simpfendorfer, 2009). Young-of-the-year and juvenile white sharks are frequently caught as bycatch (Lowe et al. 2012, Santana-Morelez et al. 2012) in artisanal

fisheries; therefore it is important to determine if these sharks are occurring in regions without protection. Regional management plans may therefore be required to conserve genetic diversity.

Due to the complexities of interpreting bulk stable isotope signatures caused by changes in diet over ontogeny and the wide-ranging habitat usage of highly migratory species, CS-SIA of amino acids (AA) has been used to differentiate between source and trophic contributions to $\delta^{15}N$ (Popp et al. 2007, Evershed et al. 2007). Differences in fractionation rates of individual amino acids allow researchers to examine if the differences in $\delta^{15}N$ values are caused by differences in baseline values or due to trophic level variation. By using CS-SIA further details on how an individual is using available habitat over ontogeny can be determined. As marine isoscapes become more prevalent the ability to define a species habitat based on isotope values of the environment will increase (Graham et al. 2010).

If satellite-tagging data were available it would allow for a comparison of time in each region with isotope value to determine the importance of a habitat to white sharks (Carlisle et al. 2014). Advances in telemetry data provide valuable insight into the movement and migration patterns of a species and allow for questions to be asked at both the individual and population level (Hussey et al. 2015). Using telemetry data in conjunction with stable isotope analysis can provide details on not only where an individual is located but if that location is important in terms of foraging (Carlisle et al. 2014). In South Africa, there has been a large scale studying fitting white sharks with SPOT tags (South Africa white shark research group and Ocearch). These data provide a vast amount of detailed information on the movements and migrations of individuals over

the period of several years. Thus, there is great potential to integrate this knowledge into stable isotope mixing models and examine the importance of regional habitat to white sharks.

Elasmobranch vertebrae provide a unique opportunity to study key characteristics over an individual's lifetime and while there are large collections of vertebrae available through museum specimens or personal collections, for some populations no samples are available. The need for lethal sampling of a species for scientific purposes has been recognized (Heupel and Simpfendorfer 2010), however for many species already under protective regulations, sacrificing animals is prohibited. For individuals being fitted with a tag (satellite or acoustic), muscle, blood, and fin tissue can be sampled allowing for researchers to gain details on previous feeding and movement patterns along with genetic information for live specimens. For species with consistent movement patterns the telemetry data can also confirm results obtained by stable isotope analysis. Additionally, details on population connectivity and similarities among related individuals in foraging patterns can be elucidated through genetic analysis. A combination of the telemetry, genetic, and stable isotope analysis will aid in providing critical information to create effective management for threatened species.

REFERENCES

Andrews AH, Kerr LA (2014) Validated age estimates for large white sharks of the northeastern Pacific Ocean: altered perceptions of vertebral growth shed light on complicated bomb $\Delta^{14}C$ results. Environ Biol Fishes 98: 971-978

Beamish RJ, McFarlane GA, Benson A (2006) Longevity overfishing. Prog Oceanogr 68: 289-302

Block BA, Jonsen ID, Jorgensen SJ, Winship AJ, Shaffer SA et al. (2011) Tracking apex marine predator movements in a dynamic ocean. Nature 475: 86-90

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42: 411-440

Bonfil R, Meyer M, Scholl MC, Johnson R, O'Brien S et al. (2005) Transoceanic migration, spatial dynamics, and population linkages of white sharks. Science 310: 100-103

Cailliet GM, Andrews AH (2008) Age-validated longevity of fishes: its importance for sustainable fisheries. In: Tsukamoto K, Kawamura T, Takeuchi T, Beard Jr. TD, Kaiser MJ (eds) Fisheries for Global Welfare and Environment: Memorial Book of the 5th World Fisheries Congress. TERRAPUB, Tokyo, Japan, pp 103-120

Carlisle AB, Goldman KJ, Litvin SY, Madigan DJ, Bigman JS et al. (2014) Stable isotope analysis of vertebrae reveals ontogenetic changes in habitat in an endothermic pelagic shark. Pro Roy Soc B 282: 20141446

Cliff G, Dudley SFJ, Jury MR (1996) Catches of white sharks in KwaZulu-Natal, South Africa and environmental influences. In: Klimley AP, Ainley DG (eds) Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, pp 351-362

Compagno LJV (2001) Sharks of the world: An annotated and illustrated catalogue of shark species known to date. Bullhead, mackerel and carpet sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Cat Fish Purposes No. 1 2: 1-269

Cortés E, Brooks EN, Gedamke T (2012) Population dynamics, demography, and stock assessment. Carrier JC, Musick JA, Heithaus MR (eds) Biology of Sharks and Their Relatives. CRC Press, Boca Raton, pp 453-485

Dean MN, Summers AP (2006) Mineralized cartilage in the skeleton of chondrichthyan fishes. Zoology 109: 164-168

Dicken ML (2008) First observations of young of the year and juvenile great white sharks (*Carcharodon carcharias*) scavenging from a whale carcass. Mar Freshw Res 59: 596-602

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD, Kyne PM et al. (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3: e00590

Evershed RP, Bull ID, Corr LT, Crossman ZM, van Dongen BE et al. (2007) Compound-specific stable isotope analysis in ecology and paleoecology. In: Michener RH, Lajtha K, (eds) Stable Isotopes in Ecology and Environmental Science. Blackwell Publishing Ltd, Malden, MA, pp 480–540

Francis MP, Duffy CAJ, Bonfil R, Manning MJ (2012) The third dimension vertical habitat use by white sharks, *Carcharodon carcharias,* in New Zealand and in Oceanic and tropical waters of the Southwest Pacific Ocean. In: Domeier ML (eds)

Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 319-342
Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: West JB, Bowen GJ, Dawson TE, Tu KP (eds) Isoscapes: Understanding movement, pattern, and process on earth through isotope mapping. Springer, New York, pp 299-318
Gubili C, Bilgin R, Kalkan E, Karhan SU, Jones CS et al. (2010) Antipodean white sharks on a Mediterranean walkabout? Historical dispersal leads to genetic discontinuity and an endangered anomalous population. Proc R Soc B 278: 1679-1686
Hamady LL, Natanson LJ, Skomal GB, Thorrold SR (2014) Vertebral bomb radiocarbon suggests extreme longevity in white sharks. PLoS ONE 9: e84006
Heupel MR, Simpfendorfer CA (2010) Science or slaughter: need for lethal sampling of sharks. Cons Bio 24: 1212-1218
Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314-326
Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE et al. (2012) Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. Oecologia 169: 395-406
Hussey NE, Kessel ST, Aarestrup K, Cooke SJ, Cowley PD et al. (2015) Aquatic animal telemetry: A panoramic window into the underwater world. Science 348: 1255642
Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fish 95: 53-63
Kim SL, Tinker MT, Estes JA, Koch PL (2012) Ontogenetic and among-individual variation in foraging strategies of northeast Pacific white sharks based on stable isotope analysis. PLoS ONE 7: e45068
Kinney MJ, Simpfendorfer CA (2009) Reassessing the value of nursery areas to shark conservation and management. Conserv Lett 2: 53-60
Knip DM, Heupel MR, Simpfendorfer CA (2012) Evaluating marine protected areas for the conservation of tropical coastal sharks. Biol Conserv 148: 200-209
Lai HL, Gunderson DR (1987) Effects of ageing errors on estimates of growth mortality and yield per recruit for walleye, Pollock (*Theragra chalcogramma*). Fish Res 5: 287-302
Lester SE, McLeod KL, Tallis H, Ruckelshaus M, Halpern BS et al. (2010) Science in support of ecosystem-based management for the US West Coast and beyond. Biol Conserv 143: 576-587
Lowe CG, Blasius ME, Jarvis ET, Mason TJ, Goodmanlowe GD et al. (2012) Historic fishery interactions with white sharks in the Southern California Bight. In: Domeier ML (ed) Global Perspectives on the Biology and Life History of the Great White Shark. CRC Press, Boca Raton, pp 169-185
O'Leary SJ, Feldheim KA, Fields AT, Natanson LJ, Wintner S et al. (2015) Genetic diversity of white sharks, *Carcharodon carcharias*, in the northwest Atlantic and southern Africa. J Hered 106: 258-265
Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H et al. (2001) Sex-biased dispersal of great white sharks. Nature 412: 139-140

Popp BN, Graham BS, Olson RJ, Hannides CCS, Lott MJ et al. (2007) In: Dawson T, Siegwolf R (eds) Stable Isotopes as Indicators of Ecological Change. Academic Press, New York, pp 173-190
Porter ME, Beltrán JL, Koob TJ, Summers AP (2006) Material properties and biochemical composition of mineralized vertebral cartilage in seven elasmobranch species (Chondrichthyes). J Exp Biol 209: 2920-2928
Post DL (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703-718
Rubenstein DR, Hobson KA (2004) From birds to butterflies: animal movement patterns and stable isotopes. Trends Ecol Evol 19: 256-263
Santana-Morales O, Sosa-Nishizaki O, Escobedo-Olvera MA, Oñate-González EC, O'Sullivan JB et al. (2012) Incidental catch and ecological observations of juvenile white sharks, *Carcharodon carcharias*, in Western Baja California, Mexico, conservation implications. In: Domeier ML (eds) Global Perspectives on the Biology and Life History of the White Shark. CRC Press, Boca Raton, pp 187-198
Simpfendorfer CA, Heupel MR, White WT, Dulvy NK (2011) The importance of research and public opinion to conservation management of sharks and rays: a synthesis. Mar Freshw Res 62: 518-527
Shiffman DS, Hammerschlag N (2016) Shark conservation and management policy: a review and primer for non-specialists. Anim Conserv doi:10.1111/acv.12265
Smith SE, Au DW, Show C (1998) Intrinsic rebound potentials of 26 species of Pacific sharks. Mar Freshw Res 49: 663-678
Tanaka S, Kitamura T, Mochizuki T, Kofuji K (2011) Age, growth and genetic status of the white shark (*Carcharodon carcharias*) from Kashima-nada, Japan. Mar Freshwater Res 62: 548-556
Towner AV, Wcisel MA, Reisinger RR, Edwards D, Jewell OJD (2013) Gauging the threat: The first population estimate for white sharks in South Africa using photo identification and automated software. PLoS ONE 8(6): e66035
Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. Mar Freshw Res 49: 553-572
Wintner SP, Cliff G (1999) Age and growth determination of the white shark, *Carcharodon carcharias*, from the east coast of South Africa. Fish Bull 97: 153-169
Zhang G, Eames BF, Cohn MJ (2009) Evolution of vertebrate cartilage development. *Curr Top Dev Biol* 86*:* 15-42

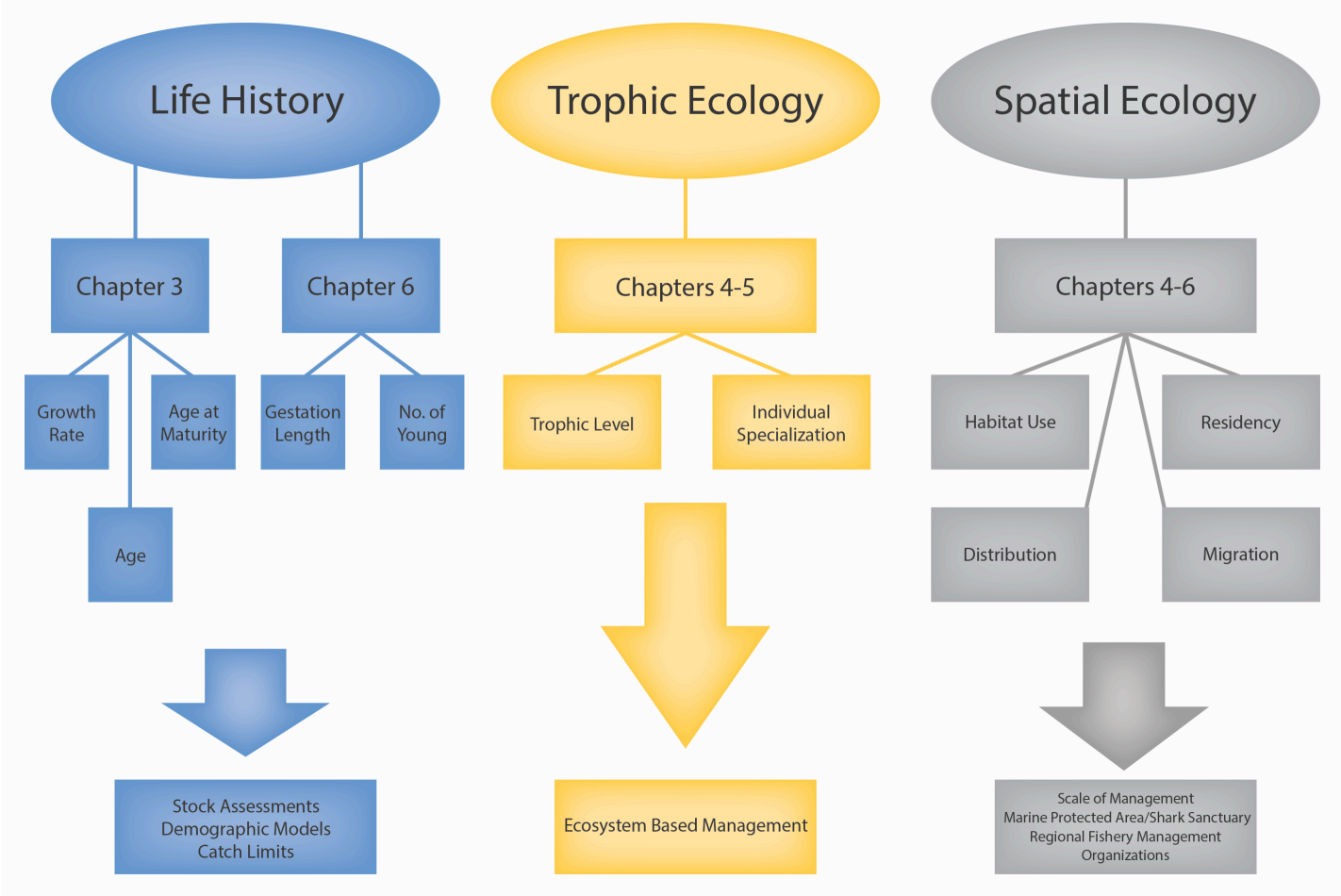

Figure 7.1 Chapter outlines of specific data deficiencies addressed for the white shark (*Carcharodon carcharias*) within this dissertation. Management policies that require data on each topic are listed below the respective arrows.

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